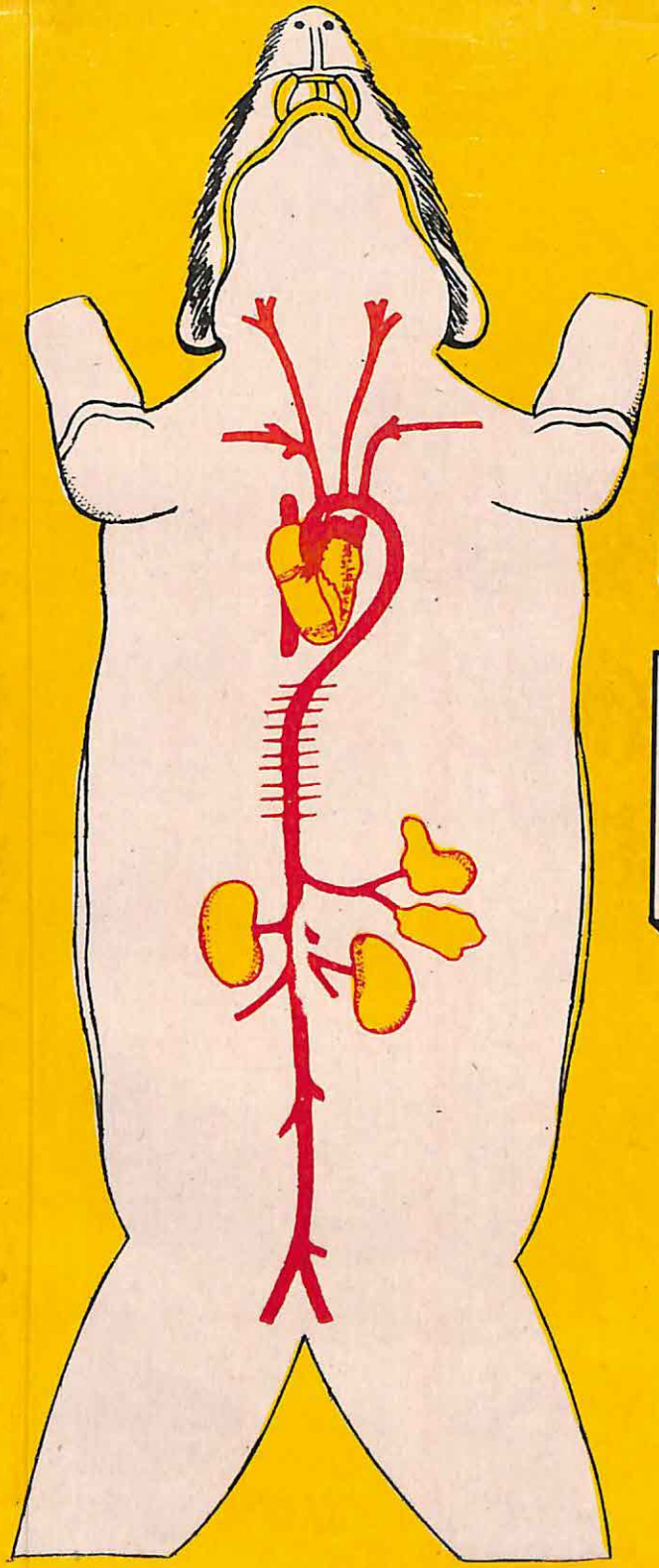

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PRACTICAL BIOLOGY

VOL. II

PITAMBAR PUBLISHING COMPANY

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PRACTICAL BIOLOGY

VOL. II FOR CLASS XII

[Strictly prepared in accordance with the latest syllabus in Biology Practicals prescribed for the All India & Delhi Senior School Certificate Examinations of the Central Board of Secondary Education, New Delhi]

By

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Preface

Biology is essentially a practical science. Gone are the days of the descriptive approach. A student of Biology or Life Sciences today has to learn to experiment, to observe, to record and to interpret the results of his observations. He has to carry out simple projects for himself and experience all the problems faced by the more sophisticated scientists.

The practical syllabus of the plus two stage includes a list of Core Experiments, which are the compulsory experiments to be done by all the students, a list of Investigatory Experiments two of which are to be carried out in Class XI and two in Class XII.

The practical text book "Plus Two Practical Biology" has been written in two volumes. Volume I covers the practical syllabus for Class XI and Volume II for Class XII. The entire syllabus has been divided into a number of Lab. exercises which have been numbered serially. These are the Core experiments. Next to be taken up are the Investigatory projects and experiments which are serially numbered separately.

Each Lab. Exercise gives the *Aim* or *Purpose, Materials required, Procedure, Directions for Observations, Guidelines for Interpretation and Precautions.* Diagrams of histology, experimental set-ups and floral diagrams etc., have also been provided to help the students. The book is intended to give guidance concerning fundamental concepts and clarity of procedure in order to develop the requisite skills in the students. Spoon feeding has been avoided and enough scope has been left to develop the creativity of the students.

Suggestions from teachers and students for improvement are welcome.

— AUTHORS

Syllabus prescribed by the C.B.S.E. for All India and Delhi Examinations for Biology (Practicals) Class XII

Students will be required to do any 10 experiments out of 14 including dissection, besides one investigatory project suggestive list of which is given in Part II.

One Practical Paper — **3 hours** — **30 marks**

PART I

(i) Core Experiments — 20 marks
(ii) Investigatory Projects — 5 marks
(iii) Class Record & Viva — 5 marks

1. Observation and making labelled diagrams of the microscopic structures of the following mammalian tissues : cartilage, bone, muscle (cardio, striated, non-striated).
2. Preparation of temporary stained (Methylene Toludino blue) slides of (a) muscle fibres of thigh muscles/pectoral muscles of frog or cockroach; (b) nerve fibre from spinol cord of frog, nerve cells from goat spinal cord.
3. Study of human and frog blood from prepared slide.
4. Dissection of rat and study (a) the general viscera and digestive system, (b) Male and Female reproductive system; (c) Arterial and Venous system.
5. Study of the action of salivary amylase on starch with special reference to denaturing agents (heat, pH and alcohol).
6. Preparation of acetocarmine stained smear of onion/garlic/legume root tip and anthers (Tradescantia or Rhoco) for the study of mitoasis and meiosis respectively.
7. Study of meiosis in squach of test of grasshopper/rat.
8. Study of variation in leaf size/pod length/seed size or number/statistical analysis, and presenstation of data in graphical form.
9. Study of nitrogen fixation in blue-green algae by protein test. Study of root nodules in a leguminous plant.
10. Study of two plant diseases of the area, identification of causal organism and alternate hosts and carriers.
11. Study of the life cycle of the silk moth/lac insect through specimens.
12. Yeast formentation and production of alcohol.
13. Testing urine for urea, sugar, albumin and bile salts.
14. Effect of antibiotics of micro organisms.

PART II

Sample List of Investigatory Projects

Note : Any one from the following in Class XI and Class XII respectively. Teachers have the freedom to add more projects in the list.

1. Study of Mendelian Traits in Pea.
2. Emasculation of a flower and selfing.
3. Population survey to identify human phenotypic characters—rolling of tongue, fused earlobes, colour blindness, etc.
4. Study of frog's/toad's ovary during breeding season to identify the states of maturation of the ova.
5. Study of the metamorphosis of toad/frog's tadpole through specimen.
6. Study of the developmental stages of Housefly/Cockroach.
7. Development of drug resistance in bacteria using antibiotics.
8. Study of coaguable and non-coaguable milk proteins.
9. Study of the effect of osmotic stress by administration of hypertonic saline in frog.
10. Locomotion in fishes, importance of different fins in balancing and steering the body.
11. Effect of alcohol, tea, coffee and nicotine on the heart beat of frog.
12. Effect of fertilizers on the rate of germination, elongation of hypocotyl and the length of root.
13. Study of the competition between seeds and crops.

CONTENTS

CORE EXPERIMENTS

INVESTIGATORY PROJECTS

CORE EXPERIMENTS

CORE EXPERIMENT 1

Aim : *Observation and making of labelled diagrams of the microscopic structures of the following mammalian tissues; cartilage, bone, muscle (cardiac, striated, non-striated).*

Material Required : Microscopes and permanent slides of the following tissues :-
Bone, cartilage, muscle (cardiac, striated, non-striated).

Note :

1. Theoretically each tissue has many identification points. All of these should not be expected to be seen in the actual slide, considering the limitations of a student microscope at this level. It is hardly possible to obtain slides that are exact replica's of the book figure. For each tissue, there are *a few key points* and more attention should be paid to these.

2. Colour of stain, (is dark or light), shape of the material, cracks and crevices or empty spaces (that are normal drawbacks of a slide due to long processes of slide preparation) *SHOULD NOT* be taken as identifying features. Students often try to make their work "easy" by such unscientific, irrational means. This must be discouraged.

CARTILAGE

1. Distinct fibrous *perichondrium* (outermost) [either on one side, two sides or all sides, depending upon size and place of cartilage].

2. Close to perichondrium on the inner side, closely *packed, elongated chondrioblasts*, with fibres in between.

3. Rest of the interior, pale coloured homogeneous ground substance,-the matrix.

4. Scattered all over the matrix, *empty lacunae, lacunae with cells* (chondrocytes) single or in gruops of 2, 3 or 4. chondrocytes *distinctly nucleated*. (Fig. 1.1).

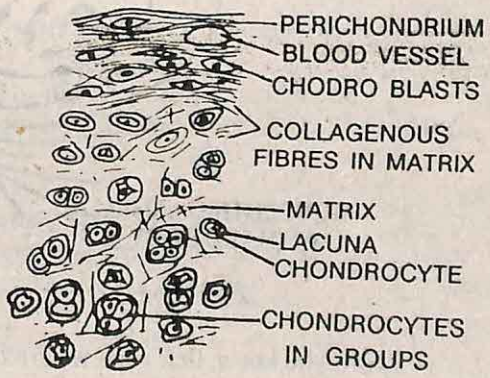


Fig. 1.1. Cartilage-T.S.

BONE

Before we look at a slide of bone tissue, we should have a clear idea, as to which part of bone we usually see in a T.S. of bone slide. A few diagrams will make this clear [Figs. 1.1 (a) (b) (c) (d)].

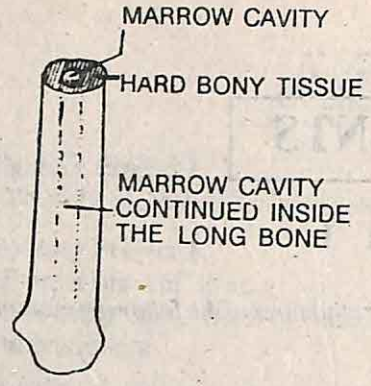


Fig. 1.2 (a)
A long bone-cut across

Note:-What we see in a T.S. of bone is a cluster of osteons as shown below.

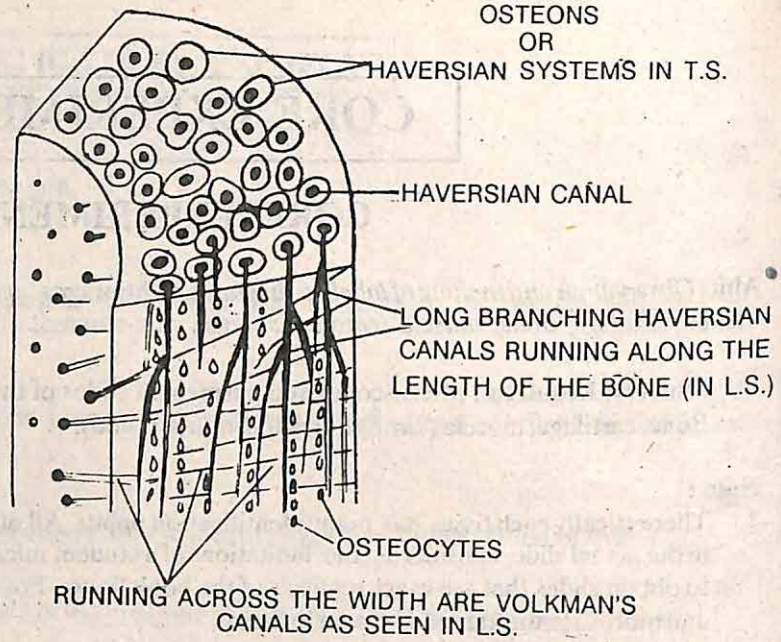


Fig. 1.2 (b)

Note:-Both canals contain blood vessels. Osteocytes in L.S. will be seen linearly arranged around Haversian canals along the length.

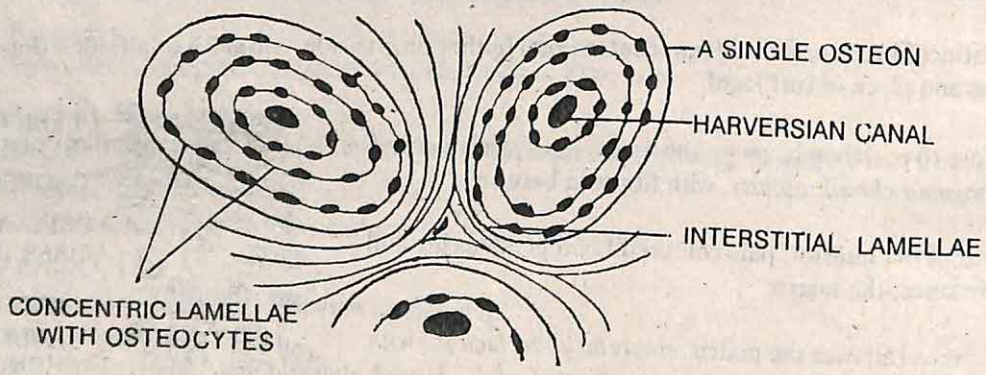


Fig. 1.2 (c)

Now you know, that what we are looking at in a T.S. of bone consists of :

1. *Concentric rings*, in distinct patches, each having a central area the *Haversian canal*. Slide of bone tissue is usually unstained, therefore cutting out little light will help. Under low power, several osteons can be seen.

2. In-between the osteons are the *interstitial lamellae*.

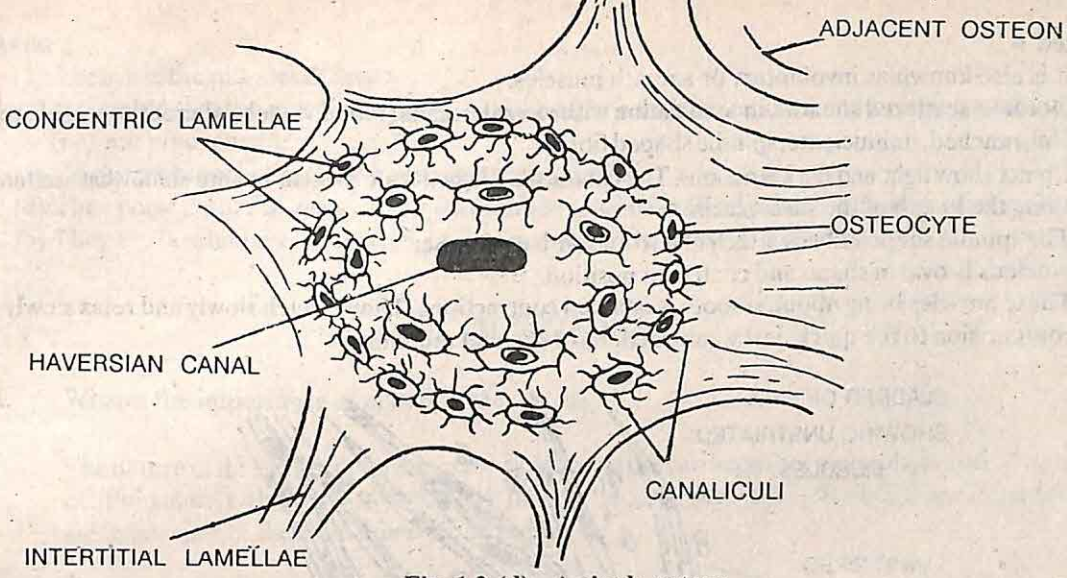


Fig. 1.2 (d). A single osteon.

3. In each osteon, *osteocytes* appear as distinct "dots" with very fine lines the *canaliculi* radiating from osteocytes visible in low power. Lacunae, however, cannot be identified in low power.

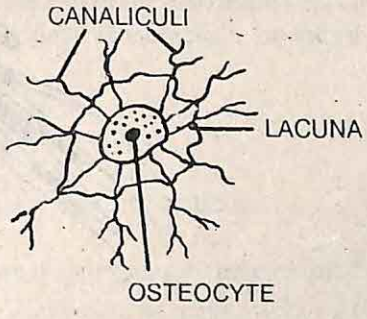


Fig. 1.2 (e)

MUSCLE Muscle tissue is in the form of long fibre like structures. Their main function is to bring about movement by contraction & relaxation. Muscle tissue is of three types, striated, un-striated and cardiac.

Striated :-

(1) It is also known as voluntary or skeletal muscle.
(2) Occur in bundles in association with skeleton.
(3) Unbranched, multinucleate fibre like cells.
(4) Show light and dark bands or striations that result due to specific organisation the contractile filaments in the fibre.
(5) The filaments are embedded in the *sarcoplasm*, covered by the membrane *sarcolemma*.
(6) The fibre may be regarded as a *syncytium* as there are many nuclei without definite cell boundaries. Each fibre is cylindrical in shape.
(7) The nuclei occur just beneath the sarcoplasm.
(8) These muscles bring about quick, elastic kind of movements and fatigue easily.
(9) Some involuntary organs that also have striated muscles are diaphragm, oesophagus.

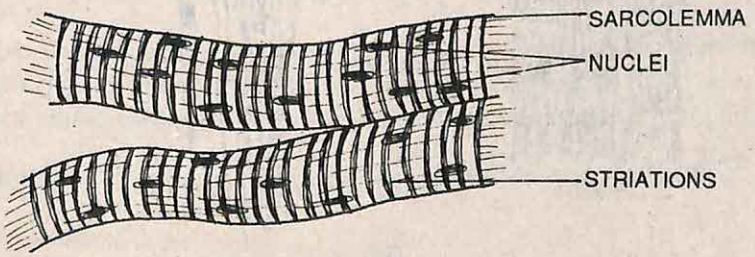
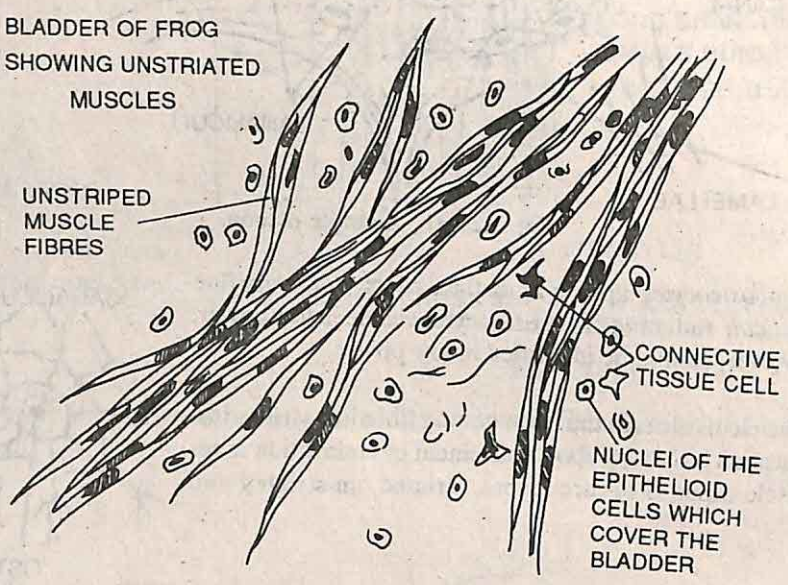


Fig. 1.3 (a) Striated muscle.

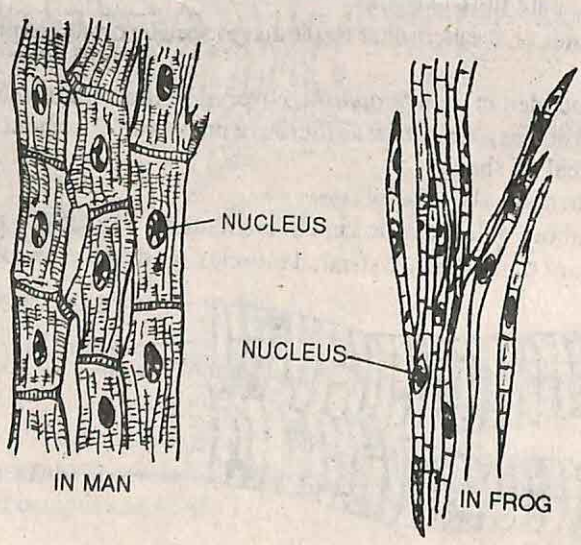
Unstriated :-

(1) It is also known as involuntary or smooth muscles.
(2) Occur in scattered sheaths, in association with viscral organs (blood vessels, alimentary canal etc.).
(3) Unbranched, uninucleate, spindle shaped fibres.
(4) Do not show light and dark striations. The contractile filaments are present but are somewhat scattered along the length of the sarcoplasm.
(5) The spindle shaped fibres attach end to end with each other.
(6) Nucleus is oval in shape and central in position.
(7) These muscles bring about, smooth, sustained contractions. They stretch slowly and relax slowly in comparision to the quick, jerky contractions of skeletal muscles.



(i)

Fig. 1.3 (b) Smooth muscles.



(ii)

Fig 1.3(c) Cardiac muscles

Cardiac :-

(1) These are the muscles of heart.
(2) Resemble striated muscles in having striations, occuring in bundles, and bringing about vigorous, (rythmic) movements.
(3) Fibres are branched and have oval and central nuclei.
(4) They possess specialized areas or discs called inter calated discs, that help in cardiac rythm.
(5) They are involuntary in function, i.e. are not under the conscious control.

QUESTIONS

Q.1. What is the importance of matrix in the cartilage ?

Ans. The nature of the matrix determines the function of the cartilage. For example, blood flows because of a fluid matrix - the plasma. Cartilage is flexible because its matrix is flexible. Bone is hard and rigid again because of the solid mineralized matrix.

Q.2. Why are bone and cartilage cells enclosed inside lacunae ?

Ans. The cells are living and must be maintained so. The non living depositions of the matrix are kept at a distance, so that the cells are not destroyed or rendered non functional. Thus, lacunae provide a protective boundary for the living cells.

Q.3. In a cartilage slide, some lacunae are empty. What has happened to the cells ?

Ans. In the process of preparation of permanent slides, cells get washed off, or are displaced.

Q.4. Give a few places of occurrence for cartilage in our body. Why are they present in these places ?

Ans. In between joints, ear lobes, nose tip, ends of ribs, heads of long bones. The functional requirements of all such places are flexibility and or reduced friction.

Q.5. Name the part of a bone, not related to skeletal function.

Ans. Bone marrow cavity of long bones. It is the haemopoetic tissue, i.e. site of R.B.C. production.

Q.6. Give some features that make bone, a complex tissue.

Ans. a) Presence of units called osteons which have concentric layers of cells and a central canal.
b) Presence of osteocytes which are highly branched and in connection with each other through canaliculi.
c) A network of canals which contain blood vessels for exchange of matter with the living cells.
d) In spite of a dry, dead external appearance bone, is a living tissue and offers tremendous mechanical support. All its functions are possible because of its complex organisation.

Q.7. What is common in all the slides of muscle tissue, whether striated, smooth or cardiac ?

Ans. Fibre like structure of muscle cells.

Q.8. Which are the peculiar features of cardiac muscles ?

Ans. These are branched, and share structural features of striated muscles but are functionally involuntary like the smooth muscles.

Q.9. Which feature of cardiac tissue is not always visible while using lab microscopes ?

Ans. Intercalated discs.

Q.10. In which of the two-striated and smooth, its easier to observe individual muscle cells ? Why ?

Ans. In the smooth muscles, because of the spindle shape of each cell and because they are scattered, and not in a bundle as in the striated tissue.

Q.11. What are the striations due to ?

Ans. They are due to the highly organised protein structures called 'myosin' and 'actin' filaments. The smooth muscles also have them but they are not visible as striations as they are not present in such an orderly fashion.

Q.12. Name a few places of occurance of smooth muscles in our body.

Ans. Walls of blood vessels, intestinal wall, loose connective tissue, bladder, uterine wall etc.

CORE EXPERIMENT 2

Aim : *Preparation of temporary stained (Methylene blue or toluidine blue) Slides of (a) muscle fibres (frog or cockroach), (b) Nerve fibre from spinal cord of frog, nerve cells from spinal cord of goat.*

Material Required : Slides, coverslips, methylene blue stain, pins, microscope and frog's thigh muscle or cockroach leg muscle.

Procedure for Striated Muscle: If the material is frog, thigh muscles can be taken. In case of a cockroach, muscle tissue can be removed from the coxa of the leg, after taking out the cuticle coxa is the fattest part of the leg and is the attached end of the leg. The slide can be made either with fresh or preserved material. Preserved material is easy to tease and separate. Pick out a piece of muscle tissue, take a small portion of it, put it on a slide that has a large drop of methylene blue stain on it. While it is in the stain, tease out the fibres with the help of common pins. Watch under the microscope, if proper stain is taken or not. Drain out the excess stain, put dilute glycerine and a cover slip.

Observation : Under low power, striated muscles appear as thin filaments, unbranched, in bundles, or isolated, [depending upon extent of teasing]. A properly stained tissue will show many tiny blue dots on the periphery-the nuclei. The striations are hair thin lines that can be seen better under the high power (Fig. 2.1).

In case of cockroach muscle, students must be able to differentiate muscles fibers from trachea which have ring-shaped thickenings. An untrained eye is likely to make a mistake. Insect muscle preparation will mostly have tracheal tubes along with it. Here are a few easy points to differentiate (Fig. 2.2).

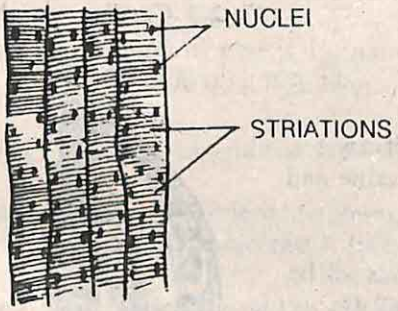


Fig.2.1. Striated muscles.

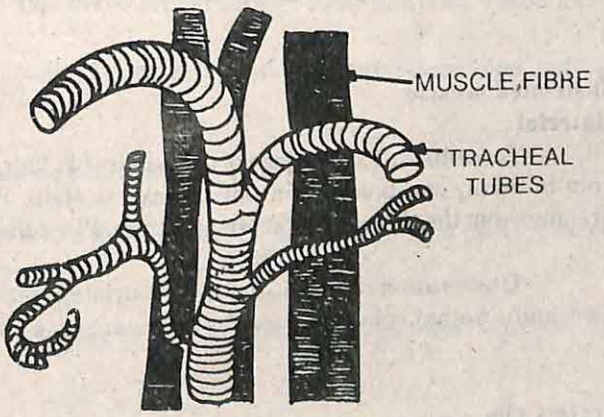


Fig.2.2. Cockroach leg muscle along with trachea.

Muscle fibre	*Tracheal tubes*
1. Are not branched.	1. Are branched.
2. They are fibres of the same thickness, often in bundles, and always in a parallel arrangement.	2. Tracheal tubes are of all sizes, some so large that one may cover up several muscle fibres. Do not lie in bundles, but form branching mesh work.

3. Nuclei stain deep blue and the rest light blue, with dark bands also as deep blue lines.	3. Do not take a proper stain, appear some what transluscent structures- absence of nuclei.
4. Striations are like straight lines across the fibres and are very very thin and very closely placed. It takes sometime before they can be focussed.	4. The so-called "striations" are actually circular thickenings not so thin as those in muscle. They can be easily seen, as dark ring like structures.

Cardiac muscle

Material Required : Same as in the case of other muscle preparations, and heart tissue of frog or sheep.

Procedure : Cut open the heart. From inside pick out a very thin section of the material and put it on a slide containing dilute nitric acid. (80 parts distilled water and 20 parts conc. HNO_3). Cardiac tissue is not very easy to tease. Nitric acid helps in softening the tissue. The material may be immersed in nitric acid for some time (30 minutes or (so) before teasing out. After this, wash the material in distilled water. On a clean slide pour 2/3 drops of methylene blue stain. Tease out the muscle fibres while it is in stain, with the help of pins. Drain out excess stain. Mount in glycerine.

Observation : Cardiac muscle will have (Fig.2.3)

(1) Branched fibres.
(2) Distinct centrally placed nucleus.
(3) Striations across the fibres.

Fig.2.3 .Cardiac muscle

Unstriated muscle

Material

Procedure : Take a piece of the urinary bladder of frog. Wash it in water, stain in methylene blue, drain out the excess stain. Put dilute glycerine and straighten out the thin membranous structure. Place the cover slip.

Observation : Long, isolated non-striated spindle shaped fibres will be seen, among other cellular structures and connective tissue fibres (Fig.2.4).

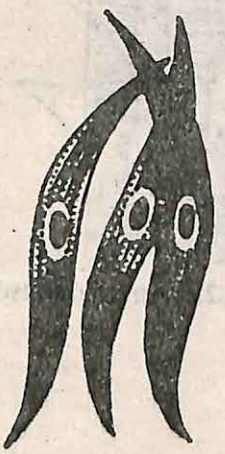


Fig.2.4 .Un-striated muscle

Nerve Cells

Material : Sheep/Goat spinal cord from a butcher's shop, rest of the material same as in above.

Procedure : Cut a small piece of the spinal cord. With the help of foreceps, pinch off a small portion of the tissue from the grey matter area (area around the central canal). [This part has more of the cell bodies of neurons] Put the material on a clean slide. Put a drop or two of methylene blue stain. Leave in stain for a

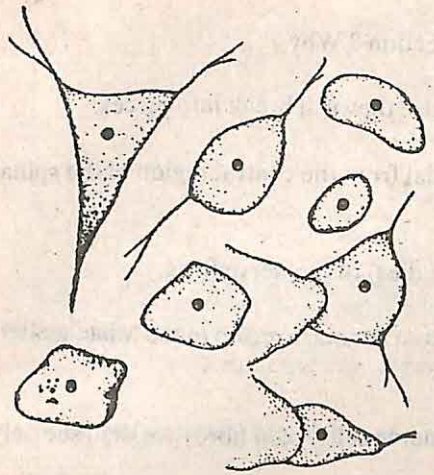


Fig.2.5. Nerve cells

couple of minutes. Drain excess stain, put a drop of dilute glycerine, place cover slip. Tap gently to disperse the matter and cells will be better visible.

Observation : Do not expect to see the complete neuron as given in books. Amidst a lot of granular matter and long, entangled masses of nerve fibres, look out for stained, large, irregular cells with deep blue nucleus and cytoplasmic processes (dendrites) (Fig. 2.5).

If one of the cytoplasmic processes appear unusually long then it may be the axon fibre.

Note : In most of these cases, the material needs to be left in stain only for one or two minutes. But students are expected to learn better from their own experience than just to stick to what the book says. This is so, because, in spite of the mentioned time, you will find that, sometimes you need to put the material for much longer time than 2 min. and at other times, the material gets stained as soon as it is put in stain. There are various reasons for such events, as proper and correct staining depends upon quality of stain, method of preparation, condition of the material etc., and these, you will agree, vary from lab to lab.

QUESTIONS

Q.1. What is the effect of using methylene blue stain in the preparation of these temporary mounts ?

Ans. Methylene blue is a genaral stain (non specific) for cells.The nucleus stains deep blue and the cytoplasm stain light blue.

Q.2. At what magnification do you see the striations in the muscle quite clearly ?

Ans. This is possible at low power of a compound microscope, i.e. at 100 times magnification. (eye piece = 10 x and objective = 10 x)

Q.3. Can you always see the nuclei and the striations at the same time ? If not, what could be the reason ?

Ans. No, sometimes we connot see these at the same focal length. The adjustments knob has to be shifted and striations are observed at one focal length while nuclei at another. This could be due to what is known as "phase differene" phenomenon. Different molecular densities absorb specific amounts of stain. Their interactions with the light rays also differ. This results in an image, where parts of the same cells are not visible at the same time with equal clarity. The striations may go out of focus (disappearing effect) when you are focusing on nuclei and vice versa.

Q.4. Teasing of muscle fibres should be done along which direction ? Why ?

Ans. Teasing should be done along the length of the fibres or else they will break into pieces.

Q.5. Why are you always advised to pinch out the nerve material from the central region of the spinal cord ?

Ans. This is because this part is rich in the cellular part (cell bodies) of the nerve fibre.

Q.6. What do you expect to see in the slide, if you take material from the outer region of the 'white matter' or the spinal cord ?

Ans. Since the outer part contains mostly the 'fibre' part of the nerve cell (Axon fibre)-we shall see only long, fibre like structures which do not take much stain.

Q.7. What is whiteness of white matter due to ?

Ans. The 'whiteness' is due to the presence of a white shinning protein sheath -the myelin sheath around the axon fibres.

Q.8. Why is 'grey matter' named so ?

Ans. This is simply because the cell body part of the nerve fibre does not have myelin sheath, and it is rich in cytoplasmic granules. In living cells, cytoplasm appears of a water colour or grey colour when unstained. The central part is rich in cell bodies, and appear gray hence the name.

Q.9. Why is the appearance of the neuron not like what you draw from the text books ?

Ans. What we observe in the temporary mount is usually the cell-body part with (sometimes) some dendrites. These cell bodies are surrounded by myelin granules all over the slide. The long axon fibres and also the dendrites are delicate strands of cytoplasm and break in the process of slide making. What we observe is actually an incomplete neuron. It is not possible to pick out neurons and lay them on the slide in their complete structure.

Q.10. Do you observe any uniformity in the nerve cells ?

Ans. No. They vary in size as well as in shape. Some appear oval, round, others appear triangular.

Q.11. Is it totally impossible to see an axon fibre in the slide ?

Ans. No. It is a matter of chance that part of the axon fibre in some cell may remain intact.

Q.12. What will you do, in case of excess of stain ?

Ans. Wash several times in water. The stain is soluble in water.

CORE EXPERIMENT 3

Aim : *(a) Study to make blood film (human blood) and to identify different types of white blood corpuscles, from prepared slides.*

(b) To make blood film of frog's blood.

A. Preparation of a blood film and observing human blood cells.

Material Required : Clean slides, dropper, blotting papar, needle, pricker, forceps, spirit, spirit lamp, Wrights stain or Leishmans stain, microscope.

How to prepare a film

Procedure

1. Keep aside two very dry and very clean slides.

2. Clean left hand middle finger with spirit and allow drying. Prick sharply with a sterilized needle or a pricker. Press. Wipe off the first drop of blood Press again. Transfer this fresh drop of blood on end of one slide.

3. Quickly place the slide with blood on some firm, smooth surface, holding in position with the thumb and index finger or left hand.

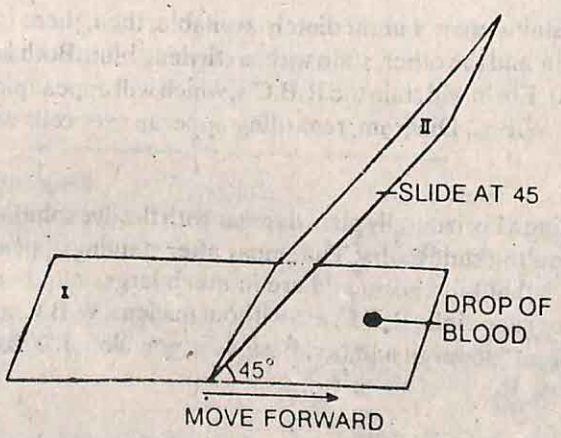


Fig.3.1 (a)

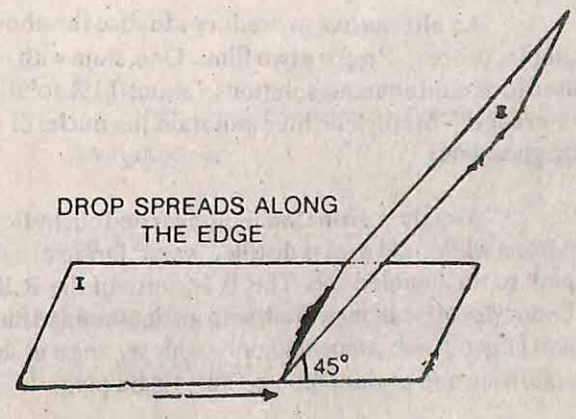


Fig.3.1(b)
Slide II moved forward until the edge touches the drop.

4. Take the second slide and place it little away from drop of blood. Move it forward until it just touches the drop. The drop spreads along the narrow edge. Now move it backward to draw out the blood in a film. The second slide should be held at an angle of 45° for obtaining a good spread out [Fig.3.1 (a) (b) (c)].

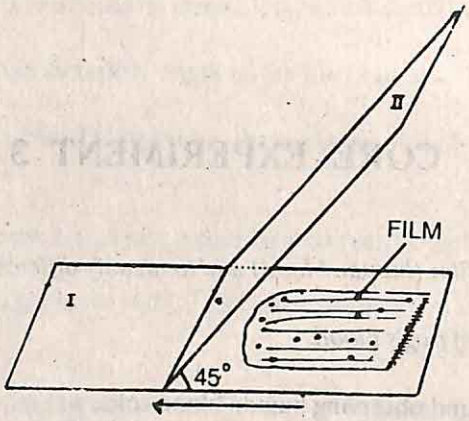


Fig.3.1(C)
Slide II moved backward and blood spreads out.

The object of making a film is to obtain a layer of blood so thin that blood cells may be seen. It an ideal film, blood cells are evenly distributed in a layer, single cell thick and neighbouring cells almost touching. Often, only a portion of the film has these characteristics.

Staining: The usual stains used are Wright's stain, Leishman stain, Jenner's stain etc. All these are a class of neutral stains and are in general known as Romanovsky stains. These stains contain a complex mixture of acid and basic dyes, dissolved in methyl alcohol.

These stains are eosin (acid dye) and methylene blue (basic). The above mentioned stains differ from each other only in their modes of preparations. The advantage of using such stains is that the different types of blood cells get stained at the same time but differentially. Differential staining is done when one wants to observe the different type of W.B.C's in the film, as well as the R.B.C's.

An alternative procedure : In case the above stains are not immediately available, then, there is a simpler process. Prepare two flims. One, stain with eosin and the other, stain with methylene blue. Both are used in diluted aqueous solutions, (about 0.1% solution). Eosin will stain the R.B.C's, which will appear pink or orange. Methylene blue will stain the nuclei of the W.B.C. Diagram regarding apperance of cells will be given later.

Wright's stain : Staining is carried out by flooding a horizontally placed smear with the dye solution. After a while, add excess distilled water. Drain off. Allow the slide to dry. The smear after staining appears pink to the unaided eye. This is because of the R.B.C's that bind eosin and are in much larger numbers. Leuocytes appear nucleated, with nucleus stained blue or deep violet R.B.C. are without nucleus. W.B.C. are also larger in size, amoeboid or roughly spherical in shape and fewer in number. Basophilic granules of W.B.C. stain blue and eosinophilic granules stain pink.

Leishman's stain - Make a dry blood film. Cover it evenly with excess of above stain . After one minute, pour excess of distilled water and allow mixing of stain and water. After about 7 minutes, pour off the mixture and cover film with distilled water for 2 min. Again wash off this water with fresh distilled water. Allow drying.

In order to make a permanent slide, use synthetic resin, i.e. Canada Balsam diluted with xylol.

In Leishman's stain, R.B.C. appear pink, nuclei of W.B.C. appear reddish purple. Acidophilic granules pink and basophilic granules blue.

Note: Leucocytes, specially neutrophils differ in appearance and staining properties in other mammals.

Characteristic features of blood cells

R.B.C. or Erythrocytes : Round or oval in shape (Fig.3.2) . Size varies from 6.5-8.0 micron. They are bicon cave discs, central area thinner than periphery. 1.0-1.5 micron in thickness. About 5 million R.B.C. are present per c.c. of blood. In stained preparations, there is a gradual transition of the intensity of stain,- easily seen under high power.

The central lighter areas appear as tiny, bright spots even under low power.

W.B.C. or Leucocytes: Are much larger cells. Roughly spherical in fixed material but otherwise amoeboid. Have distinct nuclei with morphological differences. W.B.C. also differ in their granular or agranular nature or cytoplasm. On the basis of these criteria, they are classified as follows :

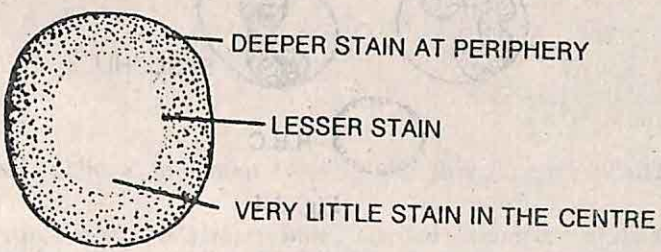
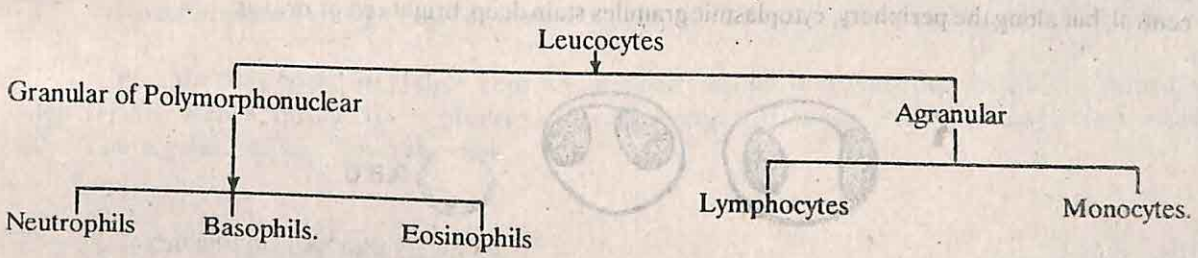


Fig. 3.2.



(Figs. 3.3, 3.4, 3.5, 3.6 and 3.7)

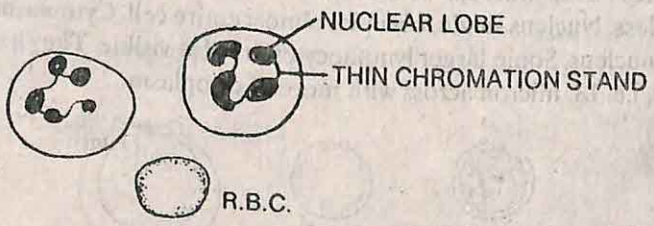


Fig. 3.3. Neutrophils

[**Note :** The term agranular is only in a relative sense. In comparison to the many granules of granular types, lymphocytes and monocytes have very few granules.

W.B.C. are numbered at about 5000 per cc of blood.]

Neutrophils : 55 to 65% of total Leucocytes.
12 - 15 micron in diameter. Cells roughly circular.

Nuclei :- Prominent, centrally placed, strain deep violet blue. Nuclei segmented into 2, 3, 4 or even 5 lobes. The lobes are inter-connected by very thin chromatin threads. Cytoplasmic granules stain deep pink or violet. Because of lot of variation regarding number of lobes, only the typical types may be studied.

Basophils :- Constitute about 0.5% of total W.B.C's. Circular in outline and measure 12 - 15 micron in diameter. Typically nuclei contain 2/3 lobes, centrally placed, stain faintly. The lobes are less distinctly separated than in neutrophils. Cytoplasmic granules stain blue or violet.

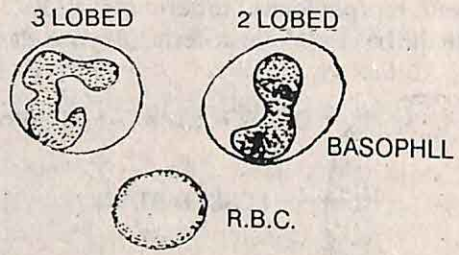


Fig. 3.4

Eosinophils : Constitute 2-3% of total W.B.C's cells are again spherical, and 12 - 14 micron in diameter. Nucleus is bilobed and lobes connected by a thin chromatin strand. Nuclear lobes very often not central, but along the periphery, cytoplasmic granules stain deep, bright red or orange.

Fig. 3.5. Bilobed eosinophils

Lymphocytes : Constitute 25 - 35% of total W.B.C's. The most common ones are small and round 8 micron across or less. Nucleus single, occupies almost entire cell. Cytoplasm, hardly visible, present only as a thin rim about nucleus. Some larger lymphocytes are also visible. They have the same features, except that they are larger, i.e. 18 micron across with more of cytoplasm.

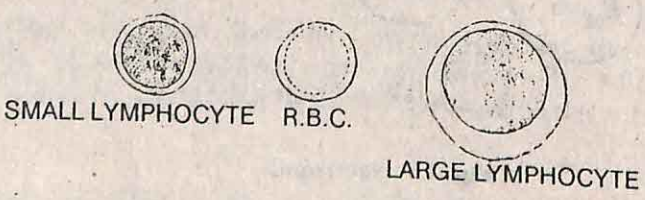


Fig.3.6

Monocytes : Constitute 3 - 10% of total W.B.C's, but vary in size and can be anything in between 12 - 13 micron in diameter. Nucleus may be central or ecentric in position. Typically the nucleus is horse shoe or kidney shaped. Cytoplasm is abundant and appears greyish.

Platelets: Are not cellular in structure, and are irregular bodies in sizes of 2 - 4 micron and therefore difficult to focus. They simply appear as specks, often clumped into irregular masses lying upon blood cells. Individually they are oval in shape with blue violet granular material in the centre. Visibility of platelets depends upon magnification available. They may be seen at (40 x10) magnification, and need to be adjusted in a plane of focus different from that of the other blood elements. Often, thread like processes extend from them and then are easily located.

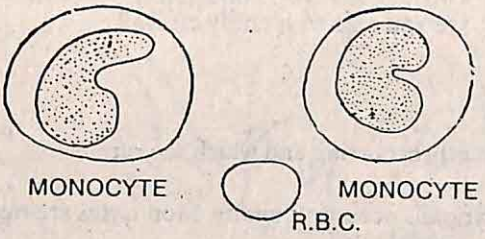


Fig.3.7

A. Frog's Blood

Material Required : Same as in human blood, except that a freshly killed frog is needed.

Procedure : Puncture the heart of a freshly killed frog and without delay take a drop on a clean slide. Make the dry blood film using the same procedure as mentioned before.

No differential staining is needed here. Frog's blood film is stained with 0.1% mehtylene blue.

Pour the stain on the dried slide, kept in an inclined position. Make sure the whole film is bathed in stain (fig. 3.8). Repeat this 2/3 times, observe under microscope. If it is over stained excess can be washed off by pouring distilled water over the slide.

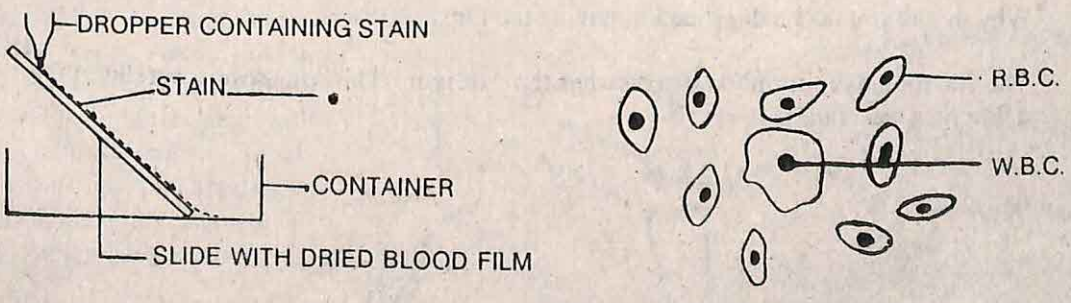


Fig.3.8

Frog's R.B.C., are biconvex or oval and with a distinct nucleus that stains deep blue and is centrally placed. W.B.C, are irregular or roughly spherical, larger and nucleated (Fig.3.8).

QUESTIONS

Q.1. What magnification is required to observe and identify the W.B.C. ?

Ans. This is done under high power of a compound microscope, i.e. at 400 times magnification. (40 x = objective lens and 10 x = eye piece lens)

Q.2. You should lose no time in preparaing the blood film. Why ?

Ans. Blood will clot and it will not form a film.

Q.3. Which types of W.B.C. are you able to identify easily ?

Ans. The lymphocytes.

Q.4. Which types are frequently occurring and which are rare ?

Ans. Lymphocytes and neutrophils occur frequently. Monocytes are rare.

Q.5. Why is there lesser stain in the centre of the R.B.C. ?

Ans. Cytoplasm which takes up the stain is present only in the peripheral part of the R.B.C.

Q.6. Differentiate neutrophils, eosinophils and basophils.

Ans.
Neutrophils - 4 or more distinct nuclear lobes connected by thin chromatin thread.
Eosinophils - 2 lobes with a chromatin thread connecting them.
Basophils - 3 lobes with thick chromatin connections.

Q.7. Where should you prick - the tip of the finger just next to the nail or on the rounded top part of the finger ?

Ans. On the rounded top part. Pricking just in front of the nail is painful as the nerve endings are present there.

Q.8. Why should you take a dry slide for making the film ?

Ans. The film spreads uniformly and sticks when the slide is dry. This is not so on a wet slide. (Try making a film on a wet slide).

CORE EXPERIMENT 4 (A)

Aim : *Dissection of Rat and study of the general viscera, and digestive system.*

Dissection of Rat

External differences, Male and Female Rats.

Place the animal on its back, in the dissection tray. The ventral side faces you. Observe this side, for the differences [Fig.4.1(a) (b)].

Female : Nipples of the mammary glands can be located after the fur is pushed aside. These will be in single row, one on either side of the mid ventral line. Anus is a crescent shaped aperture just above the originating point of the tail. Genital and urinary apertures are placed next to each other, little above the anus. The anterior is the urinary aperture and the one below it is the genital aperture.

Male : Absence of nipples. Look at the posterior end of the animal. Two oval, large sac like structures hanging on either side of the tail, but not separate from each other. The skin cover is common. These are the testes. Above this, in between the legs, is the male copulatory organ the penis with the common urinogenital aperture at its tip. Anal aperture is beneath the testes and therefore not visible in this position.

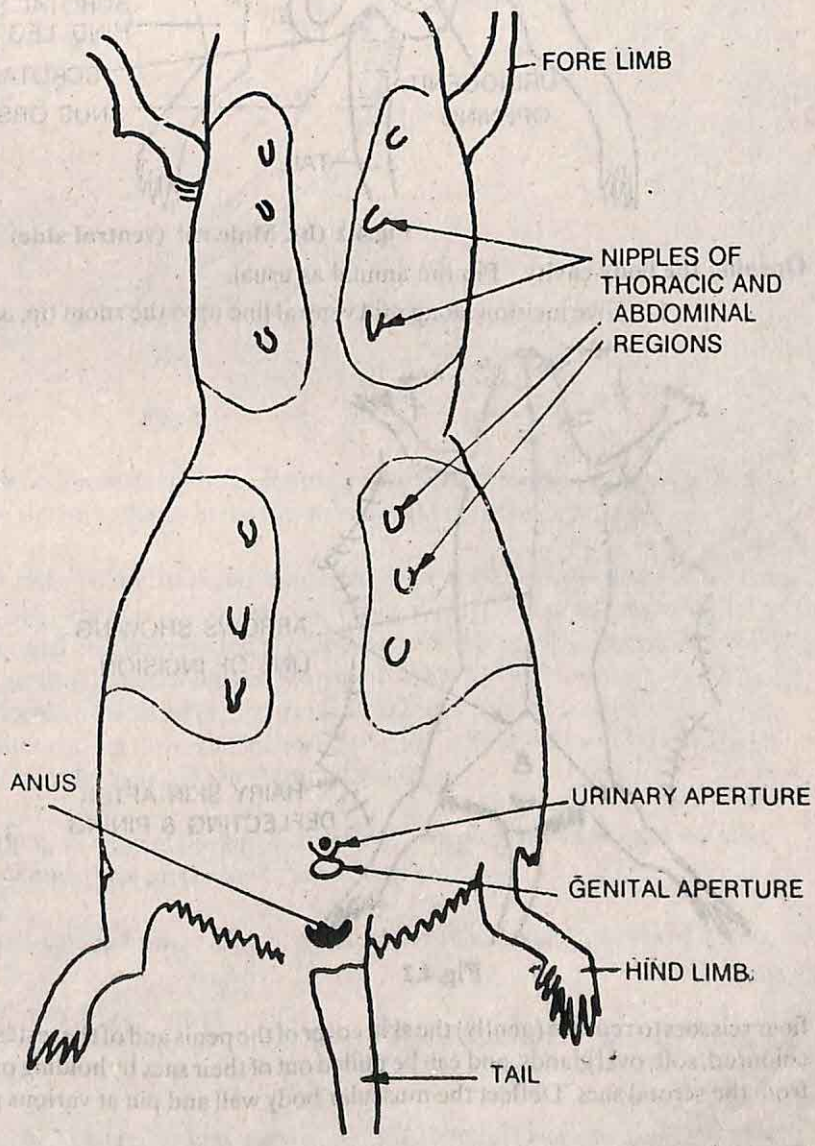


Fig.4.1 (a). Female rat (ventral side)

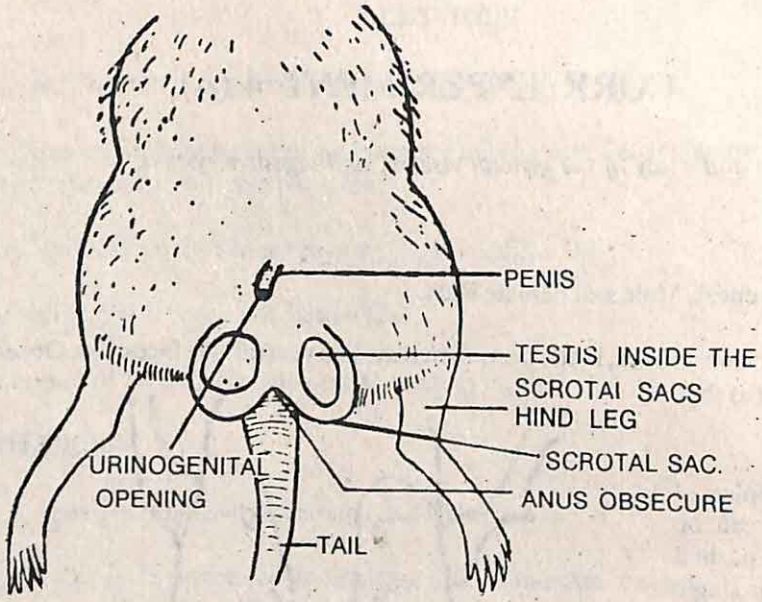


Fig.4.1 (b). Male rat (ventral side)

Opening the body cavity - Pin the animal as usual.

1. Give incision along mid ventral line upto the snout tip, along the fore-limbs, and along the ventral side of the legs. Avoid damaging the external genitalia.

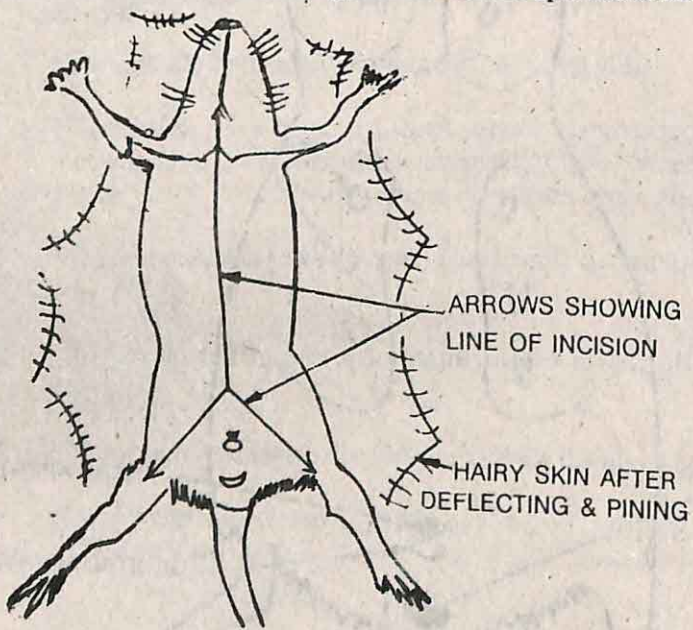


Fig.4.2

2. Rat skin is very hairy and thicker on account of the fat tissue. Therefore peeling off, of the skin will not be as easy as in frog. Tear the skin using forceful fingers and deflect to the sides. Take care in the regions of armpits, neck and groin, as blood vessels are superficial in these areas (Fig.4.2)

3. Next, give a mid line incision in the body wall. Again, take care of urinogenital organs. Body wall is continuous with the scrotal sacs (outer sac like structure containing the testes). Use finer scissors to remove (gently) the skin cover of the penis and of the testes on either side. The testes are pink coloured, soft, oval glands, and can be pulled out of their sacs by holding on to the masses of fat lobes arising from the scrotal sacs. Deflect the muscular body wall and pin at various places on either side.

4. **Abdominal viscera :** The body cavity is lined with a membrane, the *peritoneal membrane* or the coelomic epithelium. The visceral organs that are attached to the body wall, hang into the visceral cavity, by means

of *Mesentry* - which is a continuation of the peritoneal membrane. The same membrane covering one or more visceral organs together, linking is known as *omentum*, as in between stomach and spleen (Fig. 4.3).

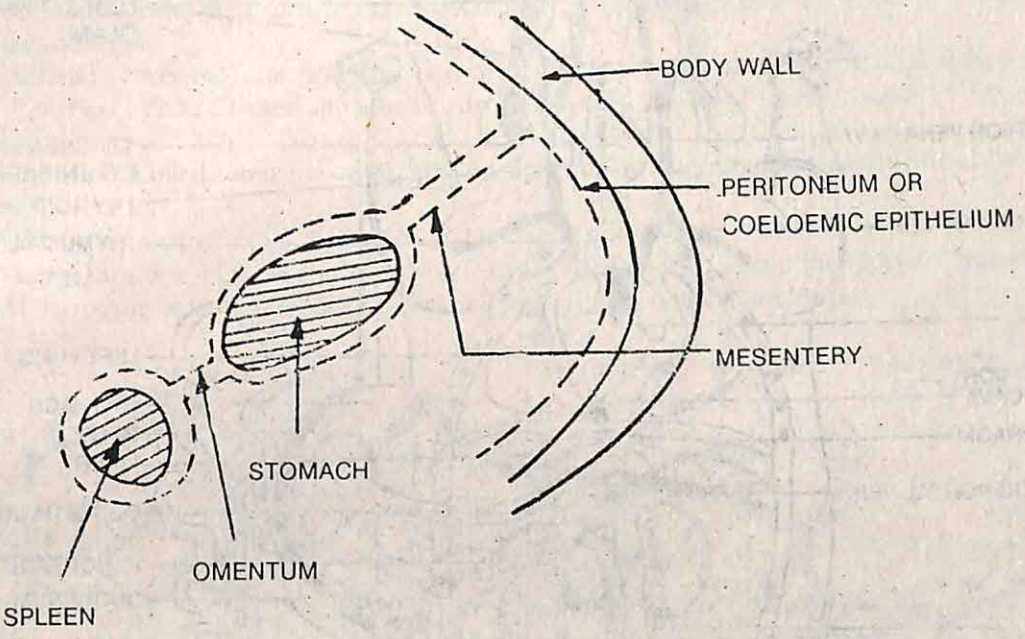


Fig.4.3

5. Elongated masses of fat in the post abdominal region, lie ventrally, and partly between folds of intestine. Pull all these masses aside to one side in order to make the abdominal organs clearly visible.

6. You can now locate liver lobes which lie below the diaphragm. Diaphragm itself is a muscular wall across the abdominal cavity. Below the diaphragm is also the stomach lying slightly on the left of the animal. Stomach is continued into the intestinal coils towards the post end, parts of reproductive organs are seen. In order to see the kidneys - the intestinal coils along with masses of fat have to be removed to one side. Kidneys are bean shaped, dark chocolate coloured organs on either side of vertebral column. Anteriorly, the heart and lungs can be seen after cutting through the thoracic basket. The lower end of the thoracic cage, i.e. the xiphoid cartilage lies on the liver lobe in the middle line.

7. Insert scissors in the mid ventral line, starting at the xiphoid cartilage, keeping the scissor tips pointing upwards, cut towards, the anterior end, through the lower jaw muscles.

8. The two halves of the rib cage now, can be further clipped off on either side to bring clearly into view, heart, lungs, trachea etc.

9. Lungs are pinkish, spongy organs and concave on lower sides to accommmodate the diaphragm, immediately below.

10. The trachea lies in the neck region. At its lower end, partly covering the heart is a large bilobed pale coloured gland the *thymus*. At the upper end is the dark red coloured, 'H' shaped gland, the thyroid. The left and right thyroids are connected across the trachea, giving it the 'H' look, (Fig.4.4).

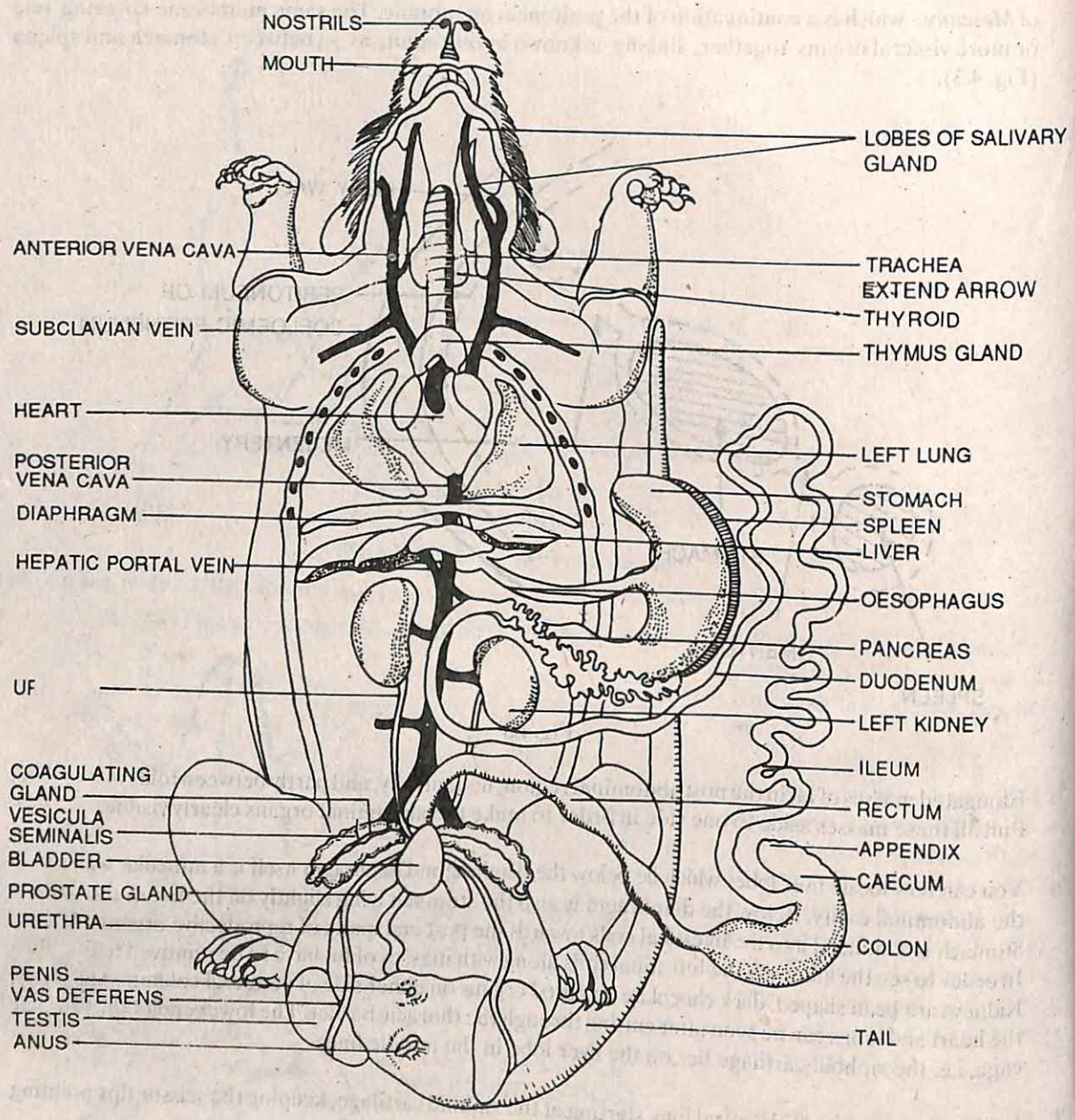


Fig. 4.4 Dissected rat to show abdominal viscera along with digestive system

Digestive System

1. Salivary glands can be seen after completing incision along the anterior region. Light coloured, soft 'pad like' structures on either side (cheek region) are the salivary glands. The three lobes are not easily distinguishable, but maximum area is occupied by the parotid (extending up to ear region) and the submaxillary lobes, as shown in above diagram.

2. In order to display the digestive system, the visceral mass of organs have to be arranged properly.

Liver : Locate the 4 lobes, partially covering the stomach, and attached to the posterior surface of diaphragm. There is a large left lobe, a median lobe with a deep cleft and the divided right lobe. There is no gall bladder in Rat.

Stomach : A sac like structure, crescent shaped at the outer edge and with an abrupt narrowing at the duodenal junction.

Spleen : A deep red (same as liver colour) crescent shaped organ, along the outer edge of the stomach.

Pancreas : Pink, diffused and lobed structure, giving a grannular texture, placed in the loop of duodenum.

Intestine : Coiled, tubular mass after the duodenum. The anterior region is Jejenum and the rest of it is Ileum.

[There is no morphological distinction, but only histological.]

Large Intestine - Has caecum, a sac like structure with a short blind end, the appendix. Next is *colon*, followed by a narrow *rectum*, often with faecal pellets, visible as nodular swellings.

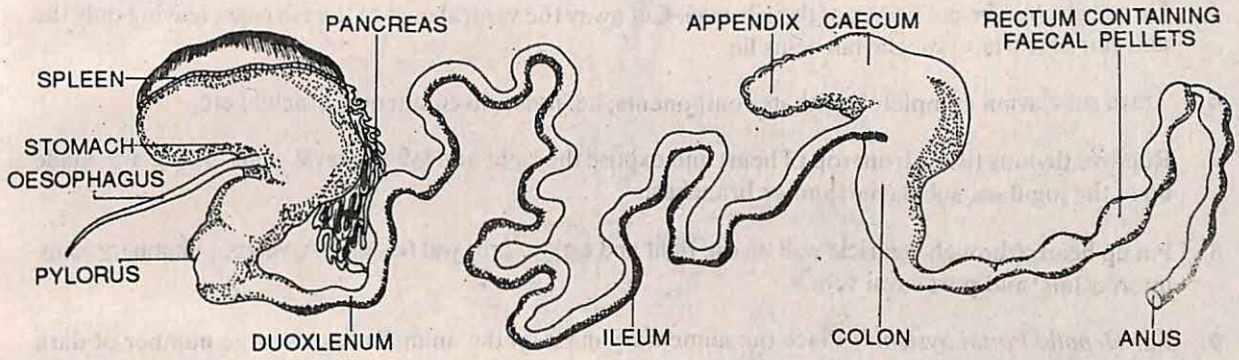


Fig.4.5 . Digestive System

[Salivary gland positions are shown in the previous diagram.]

For a good display of the digestive system, the loops of the intestines can be uncoiled by cutting through the mesenteries and fixing the parts with pins. The duodenal loop can be pinned wide apart, to display the pancreas. Too many pins should not go through liver, it being a soft tissue will break apart. The diaphragm can be trimmed suitably to spread out the liver lobes properly. Display with black paper and flag labelling. See the next experiment for the technique of black papering and flag labelling.

CORE EXPERIMENT 4(B) and (C)

Aim : *Dissection of rat and study of the veins and arteries, and male female urino genital system.*

Note: Refer previous experiment [4(A)] for procedural and material details.

For better results and better understanding, there should never be complete dependence on theoretical diagrams, while dissecting out vascular systems. This is so, becuase the exact routes followed by branches of blood vessels, while entering or leaving an organ, varies (slightly) from individual to individual, in minor degrees. Help from diagrams should be taken for a general guidance regarding the major vessels, their positions and number of branches, and respective target organs to which these vessels are connected. Every step of dissection needs application of one's creative mind. While one should obey the general rules, one should not forget that there are no hard and fast rules, when it comes to tracing a blood vessel completely, and to the best of effects. Only major blood vessels will be drawn here.

Venous System

1. Pin the animal straight and loose. Cut skin only, deflect and pin neatly.

2. *External Jugular* veins are most superficial and therefore are to be traced first of all in order to avoid damage to them. While removing the skin in the neck region, these veins are seen as very dark, thick veins on the outer edges of the neck. External jugulars are made up of inner and outer and inner edge of Parotid salivary glands and can be traced by careful deflecting of these glands, to expose the joining point. *Internal Jugulars* are thinner veins, slightly deeper and next to the trachea. These can be traced later.

3. Subclavian veins should be traced after training the external jugulars. For this, cut thoracic muscles that are Y shaped. Cut from the sternum side and lift the muscles towards the shoulder, constantly looking below the muscle flaps. Subclavian veins can be detected as very broad, dark structures lying in the shoulder region, in between muscle flaps. Once detected, cut away the top layer of muscles.

4. Now, cut through body wall, and proceed right through the sternum, and along the muscles of neck.

5. Do not stretch the cut halves of the rib cage. Cut away the ventral part of the rib cage, leaving only the anterior part where subclavian veins lie.

6. Trace subclavian completely with its components, i.e. musculo cutaneous, brachial etc.

7. Remove thymus tissue from top of heart and expose the right and left precaval veins. These are made up of the jugulars, subclavian and its branches.

8. Pin up heart, (through ventricle wall on the right and expose precaval (of left), Azygous, pulmonary veins. intercostals and post caval vein.

9. *For Hepatic Portal System* : Place the alimentary mass on the animal's left. A large number of dark coloured intestinal veins can be seen. Arrange the loops to display these. These veins join to form the hepatic portal vein, entering liver. On way to liver, one segment of intestine lies on the vein and obstructes the view. Cut this part of the intestine t get a continous view of the vein. Arrange liver lobes so as to see clearly this vein, as well as the emerging short, thick hepatic veins (3 of them) that join the post caval vein.

10. Post caval vein lies in the middle, along the length of the body. This is a dark coloured broad vessel along side Dorsal Aorta. Trace renals, lumbars, and iliacs- in that order. Ovarian veins above lumbar and testicular below lumbar veins.

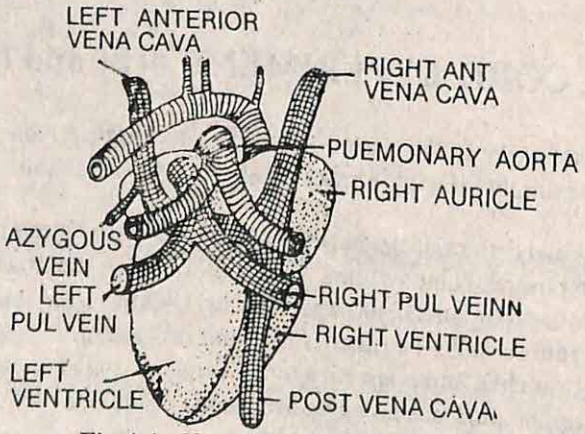


Fig.4.6 . Ventral view of heart

(*Abbreviation* : Read PUL for PULMONARY)

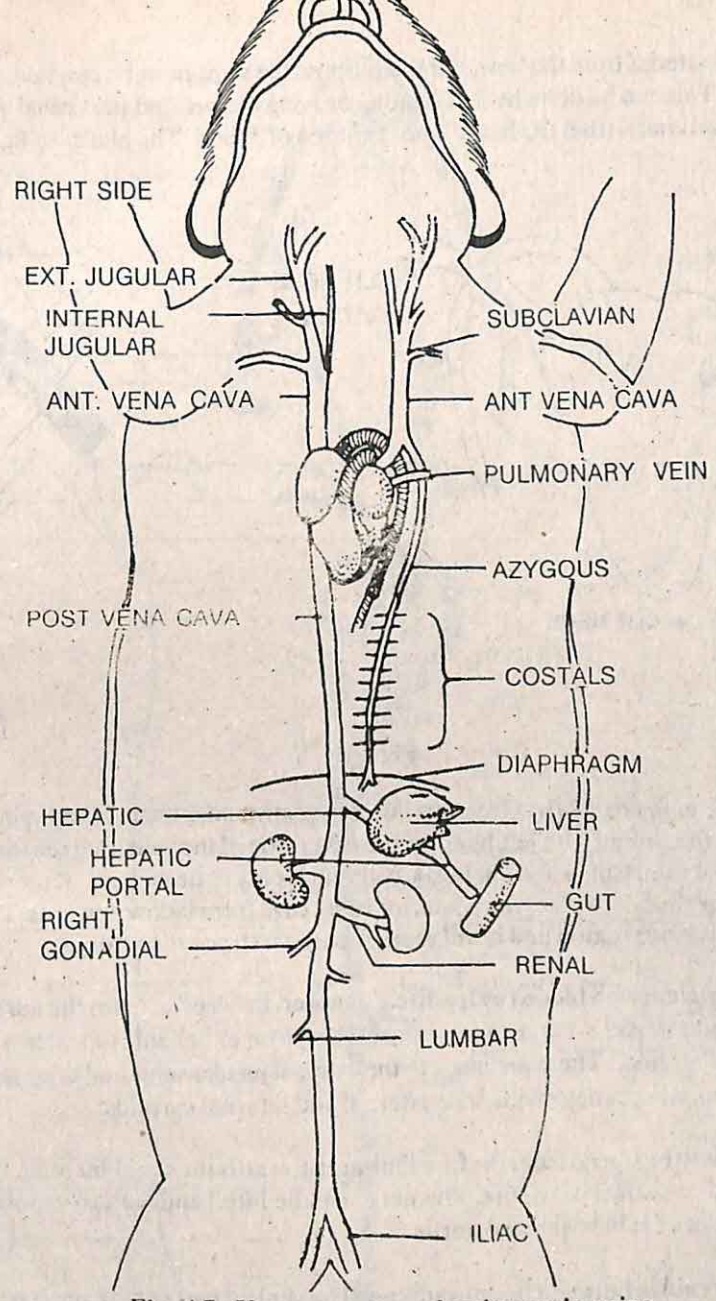


Fig. 4.7 . Venous system, showing main veins

Arterial System

1. Pin the animal straight. Cut skin and body wall, deflect the flaps of skin and body wall and pin them neatly.

2. Remove the blocks of muscle along ventral surface of neck so as to expose trachea.

3. Remove thymus gland from top of the heart. It is a loosely attached gland and can be easily removed with forceps. Detect the *aortic arch* taking a left turn and looping down below the precaval vein of this side.

4. Ligate the precaval and post caval veins. For arterial display the veins have to be removed. In the posterior part particularly the arteries and the veins run side by side. The veins being darker in colour,

hide or mask the arteries from the view. For this purpose the veins must be emptied or lifted so as to clearly see the arteries. This can be done by first ligating or tying the pre and post caval veins and then cutting them. Tying is essential so that the heart is not emptied of blood. The places of ligature are as shown in figure.4.8

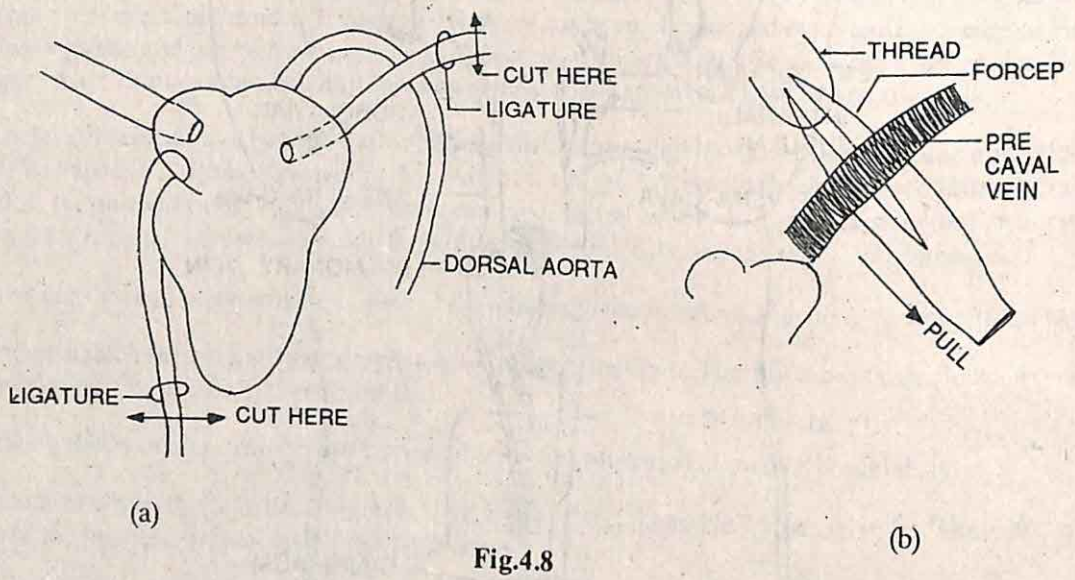


Fig.4.8

As you can see in figure.4.8 (b) place your forceps gently under the precaval vein, keeping aortic arch in view. Hold a loop of the thread with left hand on the other side of the vein. Pull the thread from below the vein and tie the precaval vein. Cut as shown. Post caval vein is easy to tie and cut, as there are no other blood vessels near it. Use your thumb to press the posterior veina cava from below upwards. This will push out the venous blood in the posterior region and it will then be easier to trace the arteries.

5. Replace the dirty water (with blood) by fresh, clean water. Proceed to trace the aortic loop downwards, where it continues as *dorsal aorta*. Remove muscle strips on either side of trachea and display carotid arteries (one on either side). These are inner to the external jugular veins and deep seated. Trace carotids upto the jaw region, where they divide into external and internal carotids.

6. Subclavian artery is to be traced from the fore limb area towards the shoulder joint. It lies in between the brachial nerve and the subclavian vein. The nerve can be lifted and cut, to expose the artery clearly. Expose joining point of subclavian and aortic arch.

7. Inter costal arteries are in between the rib muscles. The ventral part of rib cage can be trimmed away to make place for display and intercostals can be displaced in the dorsal part of the rib cage.

8. Trim diaphragm and follow dorsal aorta below it. Put the alimentary mass on the animals right although diagram shows them on the left side and spread out the loops a little. Coeliac artery, supplying stomach and upper part of intestines can be traced, by looking for it in the region that is opposite the upper edge of the left kedney. (In fact, most often coliae is in line with the adrenal gland of the left side). About a few millimeters below it, is the *Mesentric artery*. It branches several times to supply the lower parts of intestines.

9. In the same area,trace *Renal arteries* going to the kidneys and lying along the renal viens.

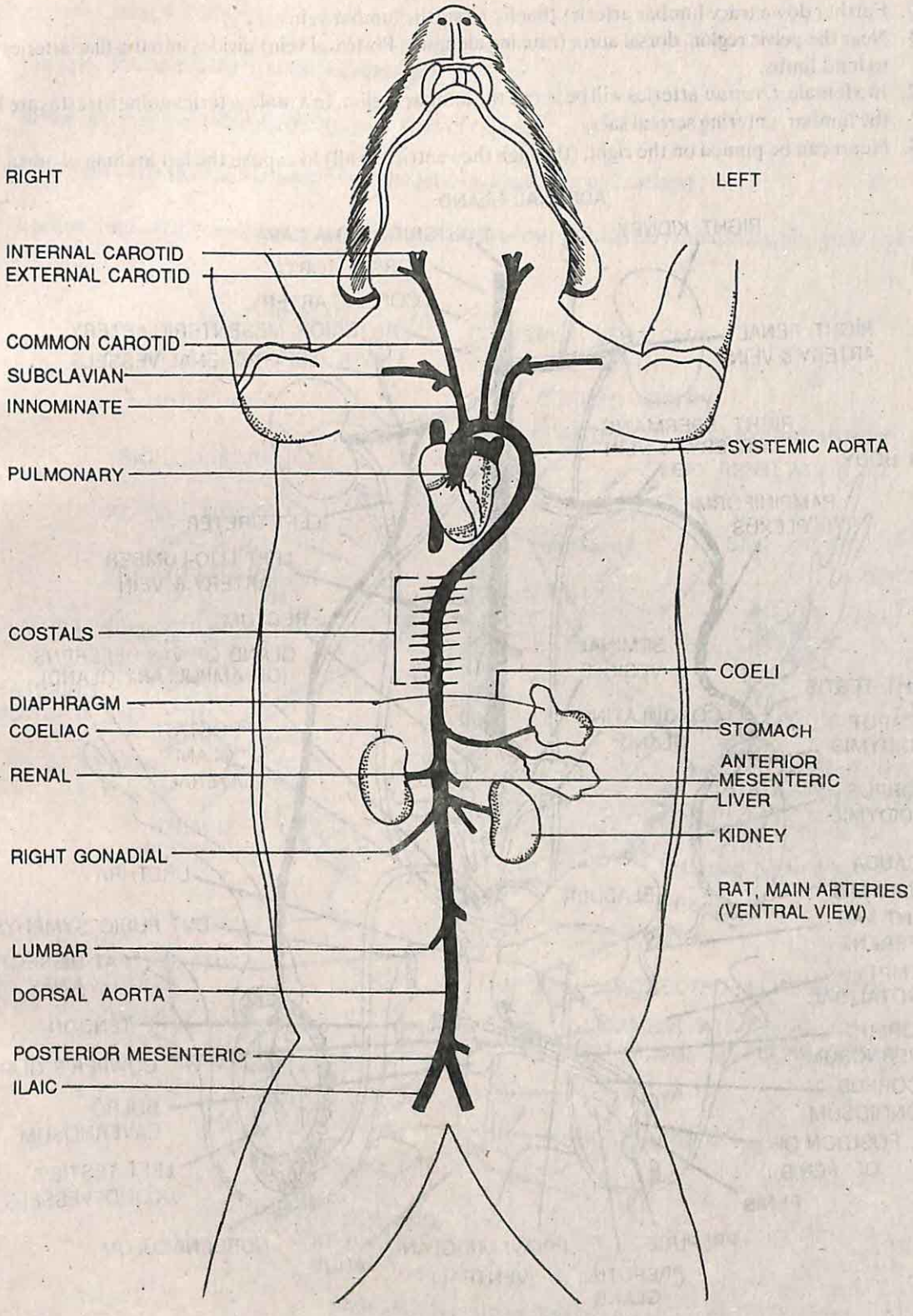


Fig.4.9 . Arteirial system

10. Further down trace lumbar arteries that lie along the lumbar veins.
11. Near the pelvic region, dorsal aorta (running alongside Postcaval vein) divides into the Iliac arteries going to hind limbs.
12. In a female, *Ovarian* arteries will be above the lumbar region. In a male arteries going to testes are below the lumbar, entering scrotal sacs.
13. Heart can be pinned on the right, (through the ventricle wall) to expose the left arching of aorta.

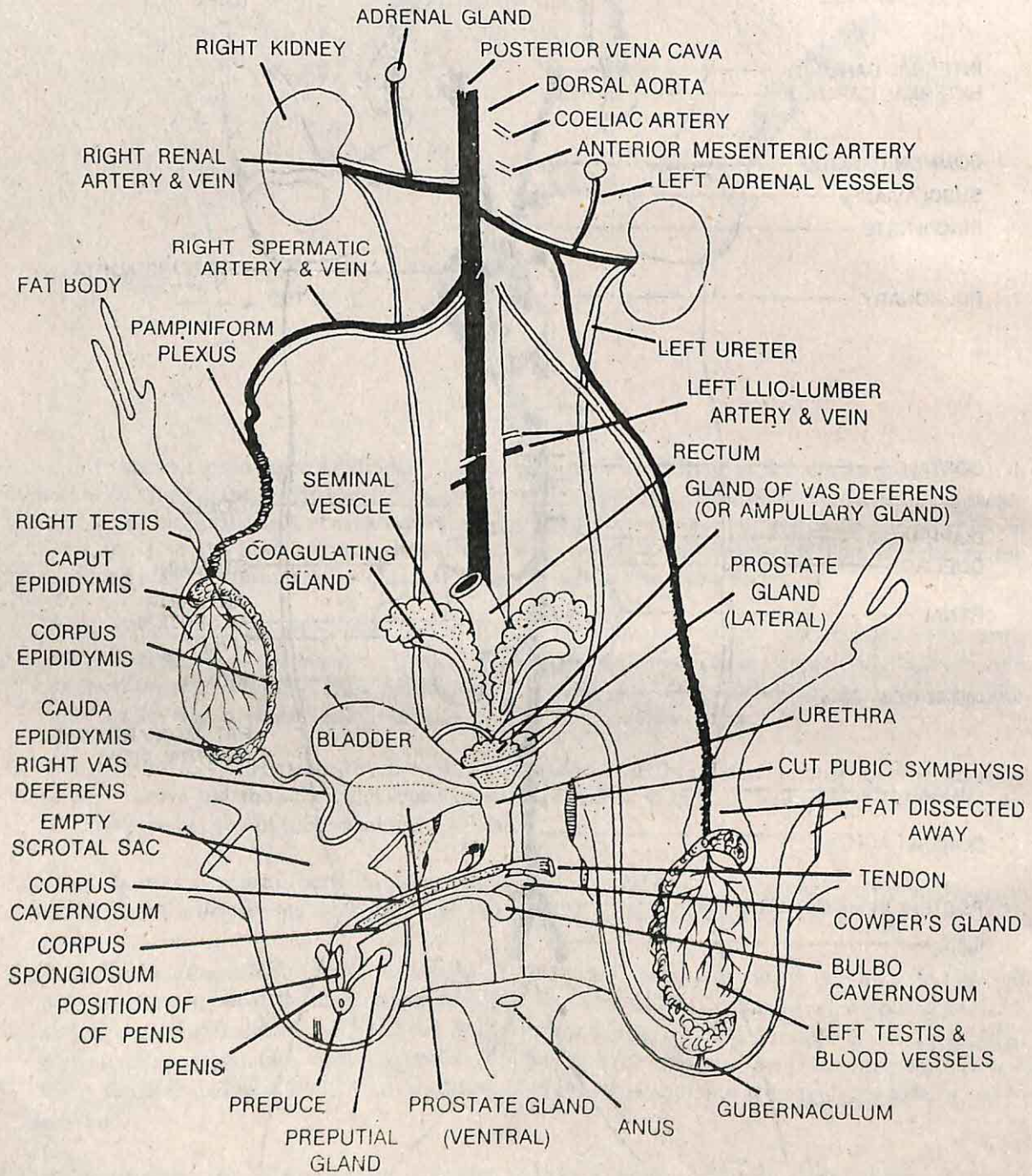


Fig.4.10 . Male system

Urinogenital System : Male, Fig. 4.10

1. Cut open the rat till anterior end of rib cage.
2. Make space by trimming diaphragm and part of rib cage.
3. Give a cut at rectal end, keeping intact the terminal part (about 2 inches).
4. Loosen alimentary canal attachments and push up the entire alimentary canal mass, pin up above kidney region.

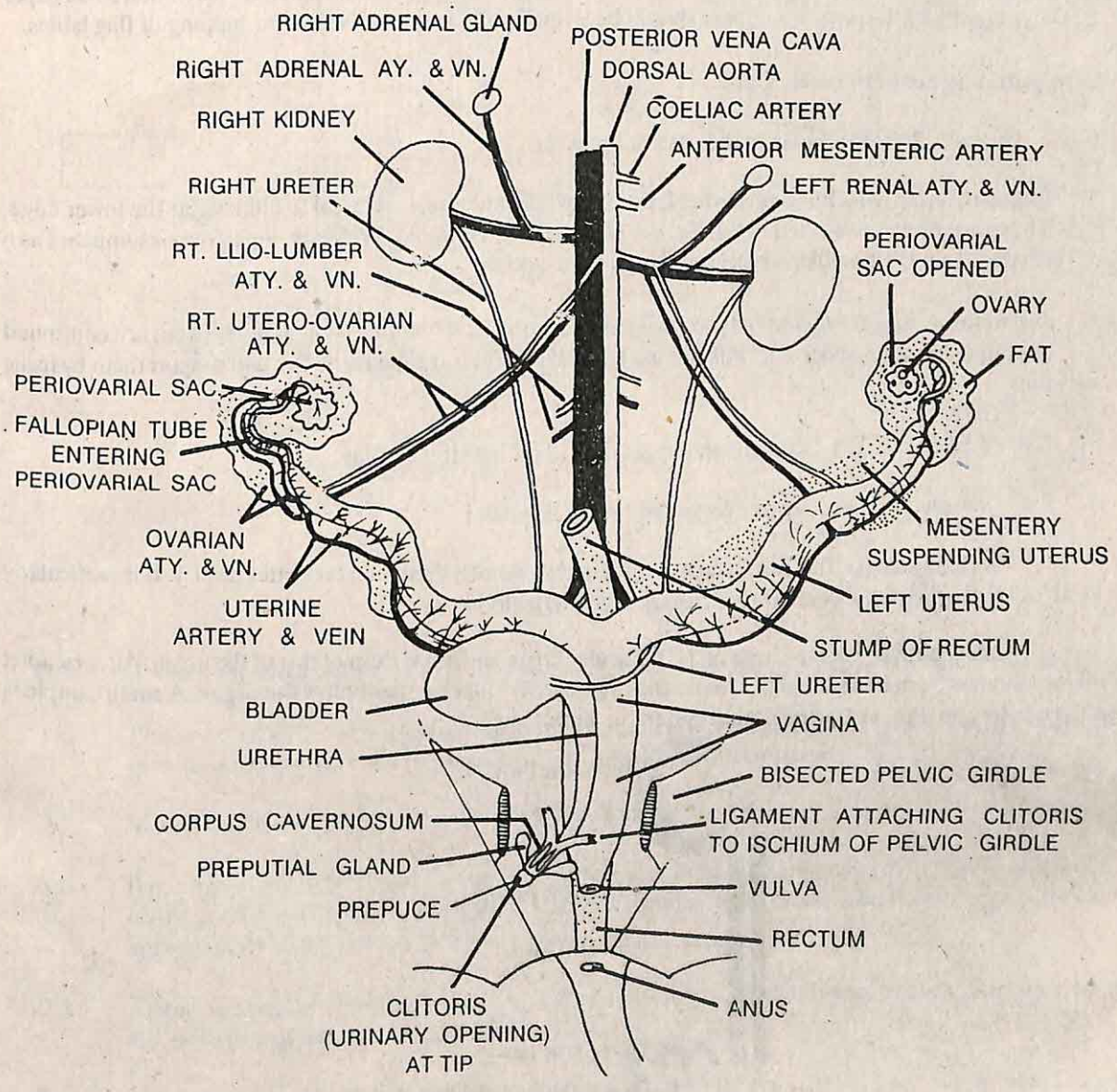


Fig.4.11. Female system

5. Pull out testes from each scrotal sac, by holding on to the fat bodies.

6. Separate (at least partially) seminal vesicles and coagulatory glands.

7. Separate the two prostates and allow them to lie on either side of the bladder.

8. Clear up all membranous attachment from below the ureters, so that black paper can be placed.

9. Cut through the skin on either side of penis for better display.

10. Put black paper under ureters, testes and the various glands. Flag label the different parts, with small paper strips and neat writing. Long pins should be in inclined positions. Avoid over lapping of flag lables.

Uriogenital System : Female, Fig. 4.11

steps 1, 2, 3, 4 same as in the case of male rat.

5. Expose ovaries, which are positioned, just below the kidneys, i.e. dorsal to kidneys, at the lower edge. These are often enveloped in fat. Excess of fat is to be trimmed. Well developed ovaries appears as a cluster of tiny blister-like structures, deep red in colour.

6. Just below ovary, are fallopian tubes, white coiled tubular structures (very small). These are continued as uterine horns on either side. Pull uterine horns slightly towards the outer side and support them by fixing pins.

7. Expose vaginal canal. Cut through the pelvic girdle for better display.

8. Put black paper on the various parts and finally flag label.

Black papering : This is a technique used in the end of a dissection for better display. It is particularly used for the urinogenital systems and also for the main blood vessels.

Cut black paper of the shape of the particular organ and a size extra of that of the organ. After clearing all membranes from beneath and around the organ, insert black paper below the organ. A small sample is shown below, in a part of urinogenital system in a male rat.

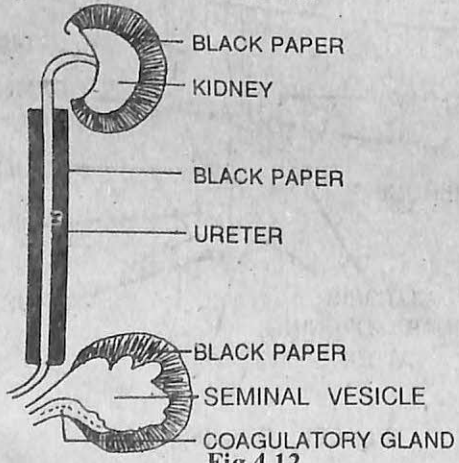
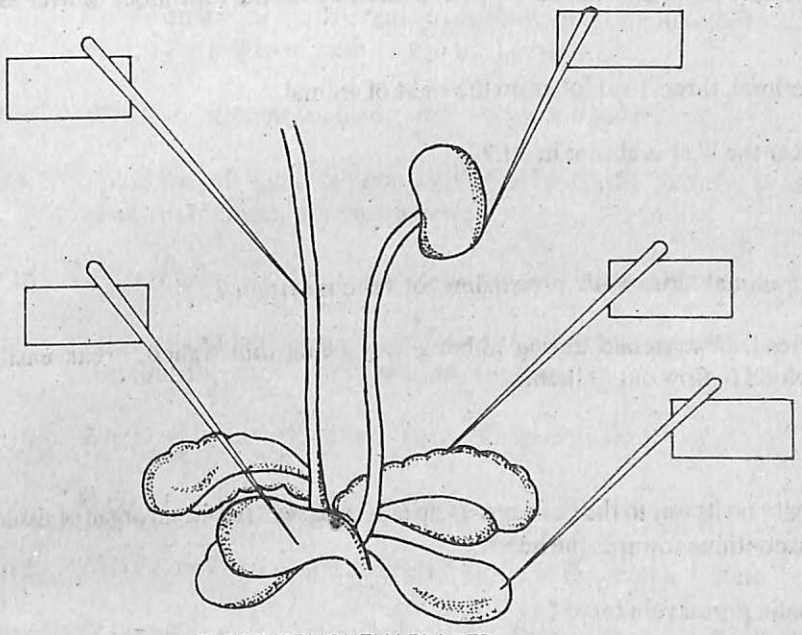


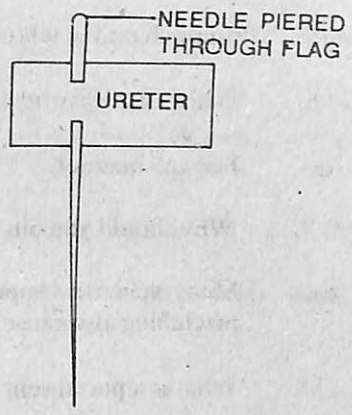
Fig.4.12

Flag labelling : This is like labelling a diagram. Cut small rectangular pieces of paper (Chart paper or thick paper) and write the various names of organs or blood vessels displayed. Take long needles and pierce the label as shown (Fig. 4.13). Insert the various flags thus made, next to the respective organ or blood vessel. A flag may be put only on one side of a paired organ. Be careful and do not place flags vertically but obliquely as shown. Vertically placed flags block the sight of the dissection. Divide your flags, half of them to be inclined on the right side and the other half on the left side.



FLAGS OBLIQUELY PLACED ON EITHER SIDE

Fig.4.13(a)



A FLAG IN A NEEDLE

Fig.4.13(b)

QUESTIONS

Q.1. A killed rat when left as such, is hard to pin. What is this due to ? What could be a remedy ?

Ans. This is so, because the muscle stiffen as they remain in a contracted state (rigor mortis). A possible remedy is to pour luke warm water and massage the muscular parts.

Q.2. In a vertebrate dissection the animal is opened along the ventral side. Why ?

Ans. It is not possible to easily cut through the vertebral column present on the dorsal side. The rib cage on the other hand can be cut through, as it is cartilagenous in the centre. The visceral organs are easily approached when a ventral incision is made.

Q.3. Among the organs - heart and lungs, oesophagus and liver, intestinal mass, kidneys & ovaries, which are more dorsal with respect to each other ?

Ans. Lungs are more dorsal than heart, oesophagus is dorsal to liver, intestinal mass is ventral to kidneys and ovaries.

Q.4. What external features will identify male from female if when they are immature i.e. nipples are not prominant and testis have not fully descended.

Ans. Common urinogenital opening at the tip of penis in male and separate urinary and genital openings (above the anus) in female.

Q.5. Which side, right or left of the animal is the stomach and three out of the four lobes of liver are situated?

Ans. Stomach on the left of animal, three liver lobes on the right of animal.

Q.6. Which storage organ near the liver is absent in rat ?

Ans. The gall bladder.

Q.7. Why should you pin the animal loose while proceeding for venous system ?

Ans. Many veins are superficial. If stretched during pinning they being thin walled, break easily. Stretching also causes blood to flow out of them.

Q.8. What is a portal vein ?

Ans. A portal vein is the one that - on its way to the heart breaks up into capillaries (inside an organ or tissue) before reuniting again to continue towards the heart.

Q.9. What function does hepatic portal vein serve ?

Ans. Hepatic portal vein is formed by the joining up of a large number of intestinal veins which bring food material (digestive end products) absorbed at the intestines. This vein enters the liver, breaks up into capillaries and reunites to emerge out as short hepatic veins that join the postcaval vein. This is essential to bring about necessary changes of the food materials in the liver cells, before they can be sent for circulation.

Q.10. What precaution should you take while pinning up the heart as part of your display ?

Ans. This pin should go through the ventricle wall only, to avoid bleeding.

Q.11. Why is it a good pracatice to ligate the precaval and postcaval veins while proceeding for arterial system ?

Ans. For a good arterial display, the veins must not be visible. In order to do that, veins should be either lifted or easier still, blood should be drained out from them. For achieving this, the main veins are ligated before cutting them so that heart retains its blood and does not collapse. Cutting of veins is possible after ligating the main veins. There after blood is allowed to drain out from the peripheral veins through these cut ends by applying finger pressure.

Q.12. Why do arteries appear lighter in colour as compared to veins ?

Ans. Arteries are thick walled with more of elastic fibres (whitish in colour) in them. Veins are thin walled

and therefore the colour of the blood shows easily.

Q.13. What is the difference in the gonadial blood vessels in case of male and female animal ?

Ans. The male gonadial vein and arteries take a longer route as their connections with the testes are outside the abdominal cavity. The female vessels on the other hand have a shorter route, ovaries being present nearer the postcaval vein next to the kidneys.

Q.14. Why is there plenty of fat around ovaries and testes ?

Ans. These are soft, delicate organs with vital functions. They are protected against mechanical shocks as well as temperature fluctuations.

Q.15. What role is played by the accessory glands in the male ?

Ans. They are endocrine glands, produce hormones that maintain the male reproductive features. They also contribute towards formation of semen.

Q.16. Where will you find mature sperms for examination ?

Ans. In the cauda epididymis part.

Q.17. Why do we cut through the pelvic girdle in the female system ?

Ans. The urethra passes through the girdle. By cutting the girdle, the birth canal is exposed.

Q.18. Is the fallopian tube a continued structure from the ovary ?

Ans. No. Ovary is independent of the tube. The fallopian tube arises very near the ovary.

Q.19. Why the latter part of the tube - has thick walls to form the uteri on either side ?

Ans. The uteri contain the developing embryos and hence have thick elastic walls.

Q.20. What difference do you know of - in the uteri of rat and human female ?

Ans. The uterus is human female is a single, triangular sac. In rat, uterus is a bifurcated structure (right and left horns) - meeting at the centre. The two uteri are tubular in shape.

CORE EXPERIMENT 5

Aim : *Study of action of salivary amylase on starch with special reference to denaturing agents like heat and alcohol and pH.*

Material Required : Test tubes, racks, pipettes, droppers, I_2 solution, saliva, water, Benedicts solution, burner, alcohol, starch solution, distilled water, 10% NaCl sol., Po_4 buffer at 6.8 pH.

Procedure

A. *Collection of Saliva*

Chew some paraffin wax or bits of rubber, until sufficient saliva is collected in the mouth. Have a clean test tube ready. Take a small piece of cotton, moisten it, squeeze out all the water and tease it out until very thin but without holes. Place this teased out cotton across the mouth of the test tube. Spit out the saliva on to the cotton. Saliva will filter through the cotton. Collect at least 2/3 ml. of saliva ; properly taken out saliva will be nearly opaque and thick. Label the tube.

B. Dilution of Saliva

Usually saliva is diluted, in order to get good results. Concentrated saliva has very active amylase and, therefore, proper gradations (if desired) are difficult to get. Saliva is diluted with distilled water :

(i) 1 ml. of saliva + 100 ml. of water
or
0.5 ml. of saliva + 50 ml. of water = 100 times dil.

(ii) 1 ml. of saliva + 50 ml. of water
or
0.1 ml. of saliva + 50 ml. of water = 50 times dil.

(iii) 1 ml. of saliva + 10 ml. of water = 10 times dil.

(iv) 1 ml. of saliva + 5 ml. of water = 5 times dil.

C. *Preparation of Substrate* : [i.e. 1% starch solution].

Put 1.00 gm of starch powder in 10 ml. of distilled water, stir well. Keep ready 90 ml. hot distilled water in 2nd container. Add small quantities from the first container to the 2nd container. Stir this well and heat (Do not boil, i.e.do not allow evaporation of water). Leave overnight. Take the supernatant.

D. *Procedure*

Prepare the following substrate mixtures, and label them as I, II, III and A.

Pour out 5 ml. of 1% starch solution in each of these tubes. Next pour out 2 ml. of PO_4 buffer at pH 6.8 in each of the test tubes. Finally, pour 2 ml. of 1% NaCl in each of the test tubes. Now place all these 4 tubes, and the saliva in a water bath at 37° - 40° temp. While this temp. is being attained prepare two sets of indicator tubes as follows :

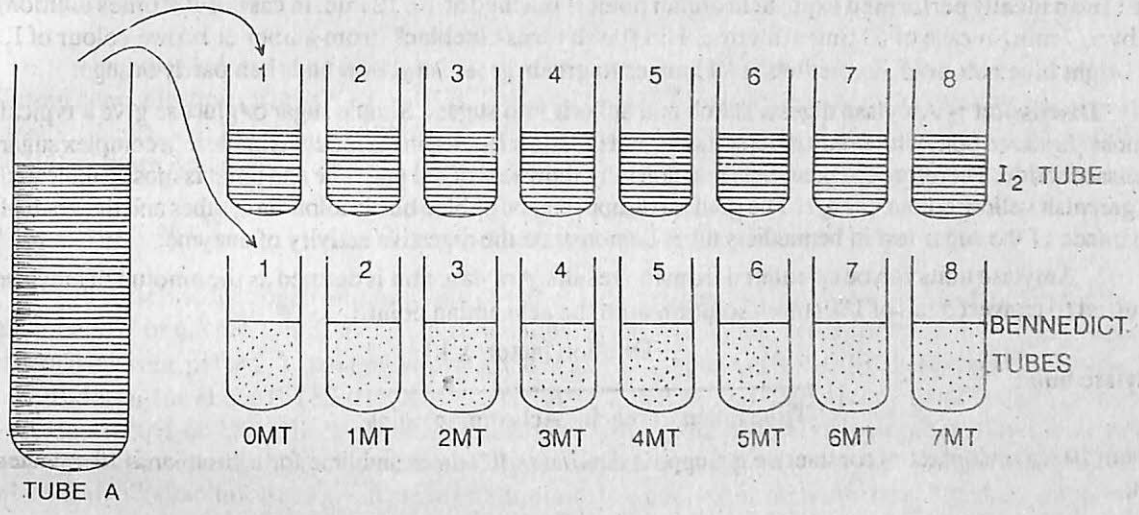


Fig. 5.1. Amylase activity

(a) about 8-10 tubes containing I_2 solution, 1 ml. each (Note that the I_2 colour should be amber brown)

(b) About 8-10 tubes containing 1 ml. each of Benedicts reagent.

Also, in a separate test tube heat strongly 1 ml. of the diluted saliva.

Once the temp. in the above 4 substrate tubes and the tube with saliva has reached 37° -40° C then while they are still in the water bath, add the following :

Tube I-Add 1 ml. of distilled water. Control experiment for A.
Tube II-Add 1 ml. of alcohol + 1 ml. of saliva (dil.) to see effect of alcohol
Tube III-Add 1 ml. of heat killed saliva (dil.) to see effect of high temperature
Tube A-Add 1 ml. of saliva (dil.) normal digestion.

Stir each of them with separate rods or in case the same rod is used, wash well each time (why ?)
Let all the the tubes remain in the water bath. Without wasting time, pour 2 drops at equal intervals of time (1 min. or 2 min.) from tube A in the indicator tubes as shown in Figure 5.1.

Tube A = Substrate mixture i.e. 5 ml. starch + 2 ml. buffer + 2 ml. NaCl + 1 ml. saliva at 37° -40° C

As shown above pour out 2 drops in both indicator tubes at regular intervals,. Note colour changes in I_2 tubes and warm up Benedicts tubes for reaction. Keep number of drops added, same for all the tubes.

Observation in A

As long as starch is present, I_2 tubes will indicate colour change. The "no colour change" point means no more starch, indicating it's complete digestion. This is the achromain point. Similarly with Benedicts, from initial negative reaction, it will gradually show a positivetest, indicating presence of sugar, resulting from starch digestion. Record the observations

Note : In an ideally performed expt., achromian point is reached at 10/12 min. in case of 100 times dilution, and by 5/7 min. in case of 50 times dilution. I_2 in starch turns blueblack, from amber or brown colour of I_2. The bright blue colour of Bennedicts sol. changes to green, greenish yellow and then partly orange.

Discussion :- Amylase digests starch and splits it into sugars. Simple sugar or glucose give a typical response (brickred ppt.) with Bennedicts solution. But the sugar formed in the mouth is maltose, a complex sugar or a disaccharide. Therefore the benndicts reaction may show less of red ppt. The end point is mostly indicated by a greenish yellow colour change. The gradual disappearance of blue black colour in I_2 tubes and the gradual appearance of the sugar test in bennedicts tubes demonstrate the digestive activity of enzyme.

Amylase units can be calculated from the results. Amylase unit is defined as the amount of amylase required to convert 5 ml. of 1% starch solution upto the achromian point.

$$\text{Amylase unit} : \frac{\text{Dilution factor} \times 10}{\text{Time taken to reach Achromian point}}$$

(Where 10 is a multiplicaton constant). e.g. Suppose dilution as 100 times and time for achromian is 10 minutes then,

$$\text{Amylase units} = \frac{100 \times 10}{10} = 100 \text{ unit.}$$

Give a thought to the various sources of errors.

Further details on amylase can be had by exposing the substrate mixture to different ranges of pH temp. etc., and by plotting graphs.

To study effect of heat on amylase activity

Enzymes are proteinous are sensitive to temperature changes. You could observe degree of tolerance to temperature variation by maintaining enzyme solution at various degrees of high and low temperatures. In the laboratory you can easily perform the above experiment at least at a high temperature and at freezing temperature.

a) High temp. : Maintain enzyme tube at boiling temp. Add this enzyme to substrate and proceed in the same manner.

b) Low temp. : Keep enzyme in crushed ice until it attains near zero temp. Proceed in the same manner.

c)Perform normal digestion of starch by amylase at 38° C temp. and let this be as the control.

Observation :-Achromian point is not reached in (a)I_2 tubes continue to turn blue black (+ve test) and Benedicts tubes continue to give -ve test, (b) Similar results may be obtained, but sometimes there may be partial +ve test in Benedicts tubes and the blue black colour of I_2 tube may be lighter ,(c) normal achromian point and +ve sugar test will be shown.

Discusstion :- Enzymes are irreversibly damaged by high temperatures. But at low temperatures they only get inactivated and if proper low temperature is not maintained, i.e. rise in temp. is allowed, then there may be little reaction in the two indicator tubes i.e. I_2 and Benedicts, and hence the partial results.

studying effect of alcohol :

Alcohol is a denaturing agent, as seen earlier. You can perform this expt. by laking at least three different concentration, of alcohol, e.g. 100% 50% and 25% Perform normal digestion without alcohol and let that be the control. Record your observations to see if alcohol exhibits amylase activily at all concentration or whether there is any partial result at low conc.

To study effect of pH

PH refers to hydrogen ion conc., and enzymes are sensitive to pH changes. Perform the control expt at near neutral or 6.8 pH. Observe the effect of adding acidic and alkaline solutions at different pH values. You could take one pH at 2, 3, or 4 (any strong acidic range) and another at 8, 9 or 10 (i.e. strong alkaline range). For considering the above PH effects, take acid Hcl at 50% or 25% and similarly any alkali Sodium or Amm Hydroxide at 50% or 25% Check pH of these solutions with the help of pH strip or other colour indicators (commercially available). Note down the pH values. Perform the expt. as per earlier instructions by adding 1 ml. of acid/alkali solution at a given pH to enzyme and then adding the above to the substrate (5 ml starch). Record your observations to indicate whether amylase can tolerate acidic or alkaline pH ranges.

QUESTIONS

Q.1. Why should we use wet cotton for collecting saliva ?

Ans. Saliva is gluey and will stick to cotton if dry cotton is used.

Q.2. Why not pour the saliva directly into the tube ?

Ans. We can pour saliva directly. (In fact that is what one is doing mostly.) But if the mouth is not clean, the saliva collected will be contaminated which may affect the results however slightly. [Certain steps such as above although not followed in practice, are a part of scientific method and one should be aware of.]

Q.3. Why chew paraffin wax and some actual food ?

Ans. Paraffin wax is not food, it will not initiate any chemical reactions in the mouth. At the same time it will stimulate the salivary glands for producing saliva. Actual food (if carbohydrate) will weaken the salivary amylase as chemical reactions for digestion will start.

Q.4. Why do different individuals have different readings for achromian point ?

Ans. Individual physiology show slight variations. The concentration of salivary amylase in the sample of saliva may vary. This in turn varies with mental, physical states of the person. Individual variations are bound to occur even in the procedural steps.

Q.5. If you reach achromian point in the first or second tube itself, what does it mean.

Ans. It means the saliva is too strong and amylase and should be diluted further.

Q.6. What is a reverse situation of above ?

Ans. Delay in reaching achromian point (i.e. I_2 tubes continue to show blue black colour) means an already weak salivary amylase. Dilution should be modified accordingly.

Q.7. What is a control experiment while demonstrating effect of temperature or toxic chemicals on amylase activity ?

Ans. The control experiment is the one showing normal digeston under optimum conditions.

Q.8. What will be a control when demonstrating normal digestion by amylase under optimum conditions?

Ans. Instead of diluted saliva, one ml. of plain water (in place of dilute enzyme) can be added to the substrate tube and then follow usual procedure.

Q.9. How is the effect of high temperature different from that of low temperature ?

Ans. High temperature brings about irreversible damage to the enzymes. Enzymes being proteins get completely destroyed when a subjected to high temperature for sufficient time. Low temperature only inactivates the enzymes. The enzymes return to normal activity on restoration of optimum temperature.

Q.10. During which months, it is easy to demonstrate digestion by amylase without having to adjust the temperature range ?

Ans. During March-April, as the room temperature is nearer the optimum temperature of 37° C.

CORE EXPERIMENT 6

Aim : *Preparation of acetocarmine stained smear of onion, garlic or any legume root tips and anthers of tradeschantia or onion.*

Material Required : Microscope, needle, slides and coveslips, acetocarmine, root tips of onion, garlic or any other legume flower buds of tradeschantia or onion (fresh or preserved) in acetic acid and absolute alcohol (1 : 3)

Procedure

A. *For Mitosis*. To obtain root tips you can keep onion bulbs in water jars, the root region dipped in water for 4-5 days. You will find tender adventitious root tips appearing from the base of the bulb. Cut these tips about 5 mm long and keep them in fixative. The best time to collect is between 10-11 A.M. Place one or two root tips on a clean dry slide. Add one drop of acetocarmine. Roll the needle over the root tips to make a smear and then cover with coverslips. You can also make a smear by covering the root with the help of needle. Heat the slide slightly till air bubbles are formed. Cool the slide. You may add a drop of glycerine if the slide dries under the coverslip. Study the slide under the microscope (high power). Look for various stages. Draw and identify each stage (Fig.6.1).

B. *For Meiosis*. Inflorescence with young flower buds of tradeschantia or onions can be collected early in the morning before 8 A.M. These can be used immediately or can be preserved in FAA (Formalin : Acetic Acid : Alcohol) after fixing in acetic acid : alcohol (1 : 3).

Take out developing stamens from four different buds of variable maturity ; mount them in acetocarmine in four different slides. Make a smear of them as described before. Study these four slides one by one for various stages in meiosis. Instead these four slides can be prepared by tour students separately and studied one by one. Draw and identify various stages(Fig.6.2).

Precautions

1. While making a smear of root tip, the cells should remain intact so as to show gradual development of various stages.

2. While making a smear from a bud the stain should be well distributed in the tissues which is possible only if the dividing cells are well separated.

3. Slight heat helps to stain chromosomes. Do not boil the stain.

Different mitotic stages.

Interphase : This is a nondividing stage. It is important to observe this stage in order to identify the features of dividing stages. The features of interphase stage are :-

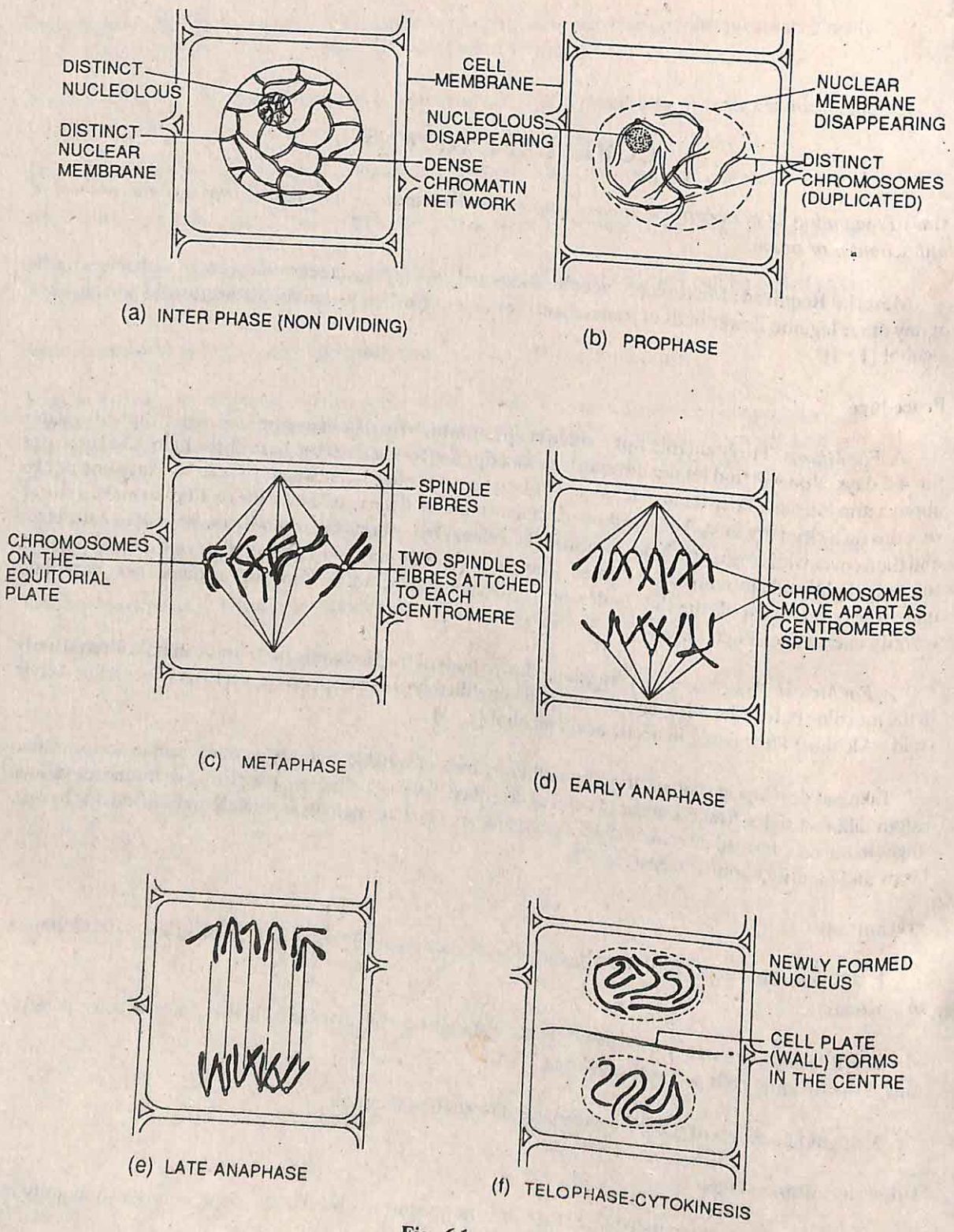
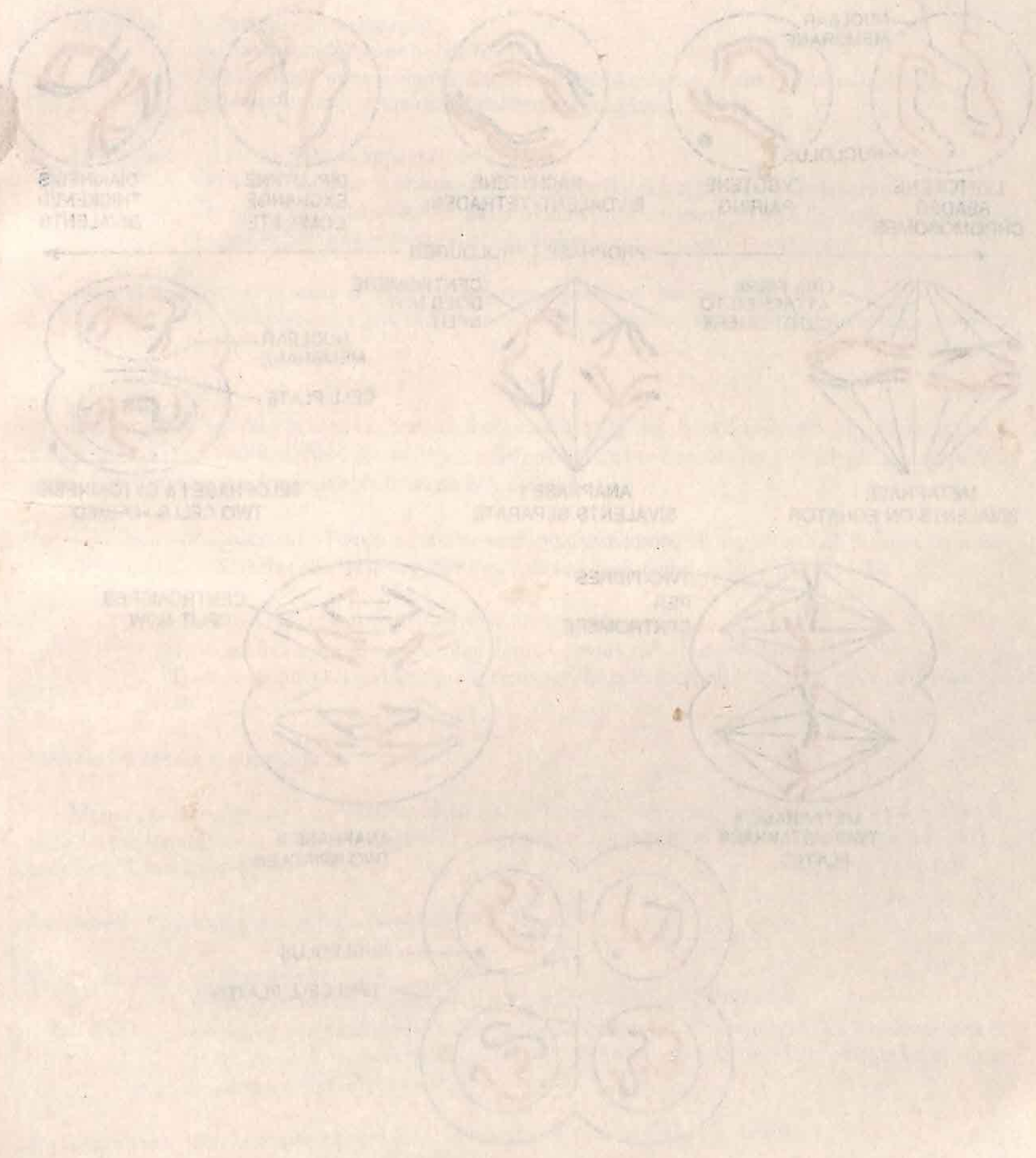
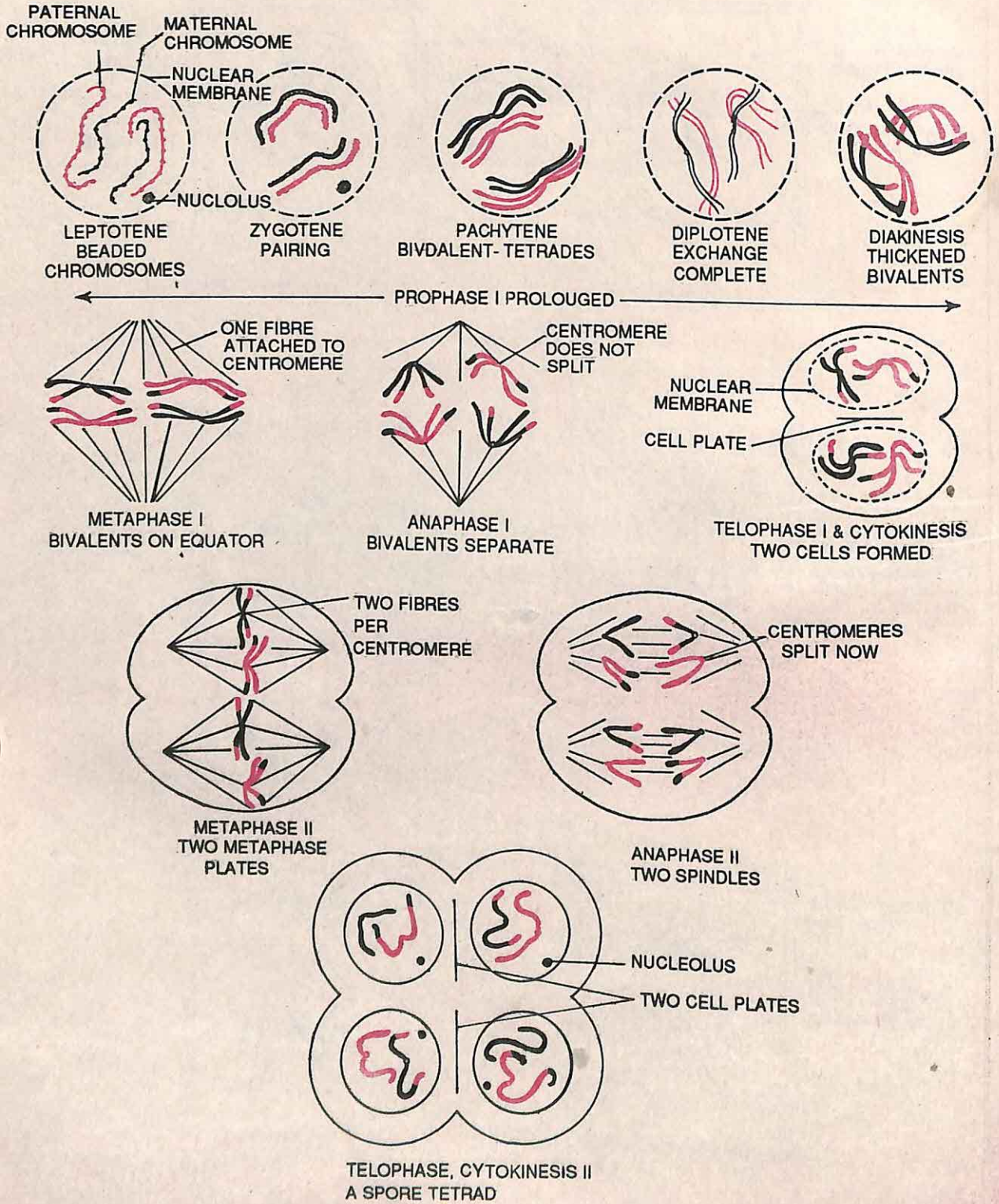

DISTINCT NUCLEOLOUS
DISTINCT NUCLEAR MEMBRANE
CELL MEMBRANE
DENSE CHROMATIN NET WORK
(a) INTER PHASE (NON DIVIDING)
NUCLEOLOUS DISAPPEARING
NUCLEAR MEMBRANE DISAPPEARING
DISTINCT CHROMOSOMES (DUPLICATED)
(b) PROPHASE
CHROMOSOMES ON THE EQUITORIAL PLATE
SPINDLE FIBRES
TWO SPINDLES FIBRES ATTCHED TO EACH CENTROMERE
(c) METAPHASE
CHROMOSOMES MOVE APART AS CENTROMERES SPLIT
(d) EARLY ANAPHASE
(e) LATE ANAPHASE
NEWLY FORMED NUCLEUS
CELL PLATE (WALL) FORMS IN THE CENTRE
(f) TELOPHASE-CYTOKINESIS

Fig. 6.1.





★ NOTE : ALL THE FOUR CELLS OF A TETRAD CANNOT BE SEEN ON THE SAME PLANE. THREE ARE OBSERVED TO GETHER, WITH THE FOURTH BEHIND THESE

(i) the nucleus can be seen as a distinct organelle.
(ii) the nuclear membrane is intact.
(iii) dense chromatin network taking a dark stain, appears almost opaque.
(iv) Nucleolus is present and may be visible, if focussed properly.

1. *Prophase* : (i) nuelear area enlarged.
(ii) nuclear membrane not distinct.
(iii) chromosomes visible as distinct thread like structures in a loose network.
(iv) nucleoplasm or space in between chromosomes visible.

2. *Metaphase* : (i) chromosomes appears thicker.
(ii) all chromosomes arranged at the equitorial plate, i.e. in the central region of cell.
(iii) spindle fibres attached to chromosomes (at centromere). These fibres are visible if good magnification and resolution are available.

3. *Anaphase* : (i)two sets of chromosomes on either side of the equitorial plate.
(ii) early typical or late anaphase may be identified on the basis of the distance between the two sets.

If the two sets are very near to each other, it is an early anaphase. But if a distinct gap is seen then it is a typical anaphase. Late anaphase shows greater distance when the two sets have reached their respective poles, and is often not distinguishable from early telophase.

Telophase and Cytokinesis : Telophase is reverse of prophase having all the interphase features returning. Cytokinesis is characterised by laying down of cell wall (cell plate) in the middle of the cell.

(i) two nuclear areas are distinctly visible at the two ends.
(ii) the nuclei show membrane, and dense network, which stains dark.
(iii) a line or cell plate is visible in the centre of the cell resulting in the formation of two smaller cells.

Different features for meiosis :-

Meiosis is completed in two nuclear divisions, with stages repeating in the same sequence. For this puspose the identification is done as meiosis I i.e. prophase I, metaphase I, and so on, and meiosis II - i.e. prophase II, metaphase II etc.

Prophase I : Prophase I is prolonged and has five substages.

a) Leptotene : (i) large nuclear area.
(ii) long coiled chromosomes (duplicated) in a delicate network.
(iii) along the length of each chromosome, darkly striained regions (chromomeres) can be seen. These densely coiled regions of the DNA are chromomeres, represent begining of condensation of chromosomes.

b) Zygotene : (i) chromosomes are more visible due to continuing of condensation.
(ii) chromomeres still visible but less so.
(iii) pairing or synapsis of homologous chromosomes occur and bivalents are formed.

(iv) because of pairing, the space in between chromosomes becomes more visible as compared to leptotene stage.

(c) Pachytene : (i) chromosomes appear thicker as duplication is complete and can now be counted as number of bivalents.
(ii) Chromomeres are not distinct any more.

(d) Diplotene : (i) bivalents are in the process of separating after exchange of genetic matter.
(ii) separation is not complete, and there are points of contacts (called chiasmata) along their lenghts.

(e) Diakinesis : (i) maximum condensation of bivalents.
(ii) paired chromosomes are joined only at ends (terminalisation of chiasma).
(iii) bivalents appear in shapes of O.U, 8 and so on are uniformly distributed in the nuclear area.

Metaphase I : (i) bivalents on the equitorial plate.
(ii) spindle fibres attached to bivalents.
(iii) Each partner of a bivalent is attached by a spindle fibre separately to their respective centromeres, i.e. the two centromeres of a bivalent face opposite poles.

Anaphase I : (i) bivalents separated.
(ii) centromeres are undivided.
(iii) two sets of chromosomes on either side, each representing the haploid set.
(iv) reduction of chromosome number achieved.

Telophase I : (i) chromosomes at respective poles.
(ii) nuclear membrane reappears.

Note : In most cases there is no distinct interphase nor prophase II in meiosis. The cell directly enters metaphase II. The 2nd half of meiosis is just like mitosis.

Metaphase II : (i) two simultaneous metaphase plates can be observed.
(ii) chromosomes in the two daughter cells arrange at equitorial plates.
(iii) centromere of each chromosome attached to spindle fibres from both poles.

Anaphase II : (i) centromeres spiit and each cell show two sets of separated chromosomes.
(ii) in all four sets chromosomes are visible.

Telophase II
Cytokinesis : (i) four sets of chromosomes are now closely packed.
(ii) nuclear membrane reappears.
(iii) two cell plates simultaneously laid.
(iv) four distinct cells or spore tetrads formed.
(v) under the microscope all the four sets can be not seen together but only 3 can be seen, the 4th one being on the other side.

QUESTIONS

Q.1. Define mitosis and meiosis.

Ans. Mitosis, also known as somatic or equational division, results into two identical nuclei with the same amount of hereditary instruction as its parental cell.
Meiosis or reductional division results into four haploid nuclei, after two sucessive divisions. In meiosis one of the division is equational.

Q.2. How do yo differentiate between mitosis and meiosis in permanent slides ?

Ans. A permanent slide showing mitosis would show all the cells of equal sizes., almost square like. Since the mitosis is shown from plant material like root-tip, the cell wall separates equal size of cells in the zone of multiplication. Number of chromosomes is the same in a somatic cell and daughter cells. Only one centromere is seen at the equatorial region for a pair of chromatids.
A permanent slide showing stages in meiosis show the cells of varying size. The meiosis can be shown from the material like pollen grains (developing) or testis squash. Distinct prolonged stages in Prophase I would be visible. In Metaphase I, two centromeres with homologous chromosomes (at chromatid stage bivalents) would be there.

Q.3. Which is the correct region of from root to for preparing mitosis slide ?

Ans. About 3 mm. from the root tip.

Q.4. Which part of the flowering bud is used for the preparation of meiosis ?

Ans. Select the flower bud (youngest ones) and remove the anther lobes under a dissecting microscope.

Q.5. How do you differentiate between metaphase and anaphase (mitosis) ?

Ans. In metaphase the centromeres are located at the equatorial plate and chromatids are facing the poles. In anaphase the centromeresh divide and move towards the polar region along with nuclear spindle. The arms of the chromosomes face towards equatorial plate. Two sets of chromosomes seen.

Q.6. Why is mitosis known as equational division ?

Ans. Because the chromosome number remains the same in the daughter cells.

Q.7. Where and when are the chromosomes reduced to half in meiosis ?

Ans. Movement of the homologous chromosomes begins at Anaphase I and they reach to the poles at Telophase I.

Q.8. What name is given to the cells of meiotic products.

Ans. Gamete.

Q.9. Why is it necessary to fix the cells in a fixative ?

Ans. By keeping the cell material in a fixative, the cell division is arrested and the cells can be studied showing diffrent stages.

Q.10. What is the composition of fixative ?

Ans. 3 parts of Absolute alcohol and 1 part of Aceitic acid.

Q.11. How can you differentiate dividing cells from other cells in a root tip smear ?

Ans. These cells are squared shaped.

Q.12. Name different phases of mitosis.

Ans. Prophase, Metaphase, Anaphase, Telophase.

Q.13. Name different phases of meiosis.

Ans. Prophase I consists of Leptotene, Zygotene, Pachytene, Diplotene, Diakinesis. Metaphase I, Anaphase I, Telophase I, Prophase II, Metaphase II, Anaphase II, Telophase II.

Q.14. Why is meiotic prophase prolonged ?

Ans. During prophase I. The duplication of DNA material is completed followed by crossing over between homologous chromatic material.

CORE EXPERIMENT 7

Aim : *Preparation of testes squash of grasshopper, and identification of meiotic stages.*

Material Required : Grass hopper testes, acetocarmine stain, slides, cover slips, needles, droppers, spirit lamps, blotting paper, microscope.

Procedure

A. To obtain testis

These can be obtained from the dealers. If these were preserved in formalin by the dealer then one must wash these with water before use.

Testis can also be obtained from the grass hopper. Following is the procedure for dissecting the grass hopper.

Peocilocerus pictus is found on AK (Calotropis) plants, after summer; they are more conspicuous during and after rains. This grass hopper is diurnal, actively feeding on the leaves, crawls, leaps and flies. Belongs to the order Orthoptera. Body is elongated about 80-90 mm, marked with yellow, blue/blue green spots and bands. The head, thorax and abdomen are very distinctly visible.

How to identify a male grass hopper i.e., external features : It has longer wings, extending beyond the end of the abdomen. Have a single pair of anal cerci and lack the black-tipped, flexed ends of terminal abdominal region (Fig.7.1).

Female : Wings leave posterior abdomen uncovered. Egg laying apparatus consists of valves that have their tips dark, hardened and projected upwards (dorsal tips) and downwards (ventral tips) (Fig. 7.2).

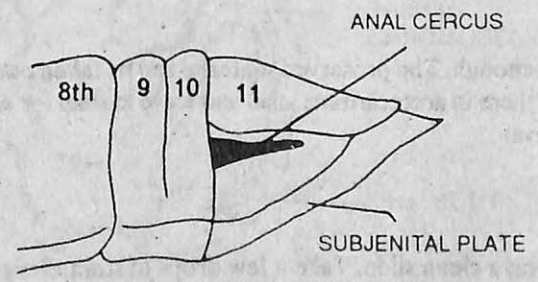


Fig. 7.1. Male abdominal tip

Fig. 7.2. Female abdominal tip

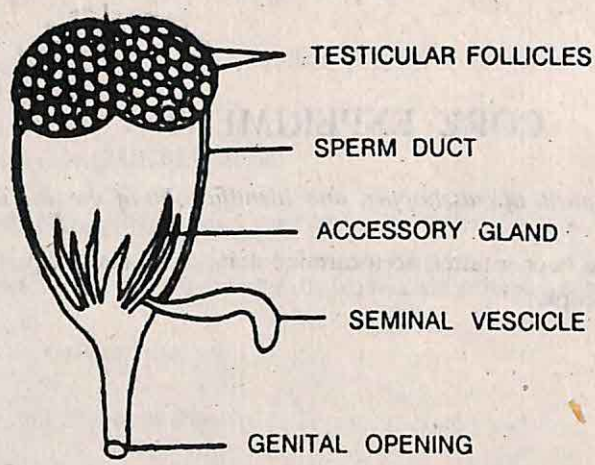


Fig. 7.3. Male Reproductive system

The anal cerci in male are longer and easily visible. Select a male grass hopper, kill in chloroform. Hold the insect with left hand and give a straight dorsal incision, along the middle region of the abdomen, upto thorax, the testis, one pair are very distinct oval bodies, lying in the 3rd to 5th abdominal segments, above the alimentary canal and embedded in fat tissue. Gently remove the fat tissue and look out for the large oval bodies, each made up of a mass of slender tubules or follicles. Each tubule has a minute duct, all of which finally join to form the sperm duct.

B. Fixing and preservation

Gently take out the testes with forceps and fix in acetic alcohol fixative or Carnoy's fixative. Let the material remain in the fixative for at least 15 minutes. Transfer to a preservative. Testis can be preserved in 70% alcohol or formalin (dilute). Every school can fix and preserve its own material which will be economical as well as more dependable.

C. Staining

One follicle per student is enough. The preserved material can be taken out on a watch glass, separated from the fat and isolated. Place them in acetocarmine stain and leave in stain for at least 30 minutes, preferably a little more time should be given.

D. To prepare the slide

Place one stained follicle on a clean slide. Take a few drops of stain alongwith it. Warm it very gently over a gentle flame. (Warming is not heating). Put a coverslip, gently press with thumb to make the squash. Drain out excess stain with blotting paper. Observe under microscope and identify the stages. Use lenses which will allow magnification upto 10 x 40, i.e. 400 times or 10 x 45, i.e. 450 times.

E. Identification of stages

The 2n no. in the male grass hopper is 19, because of the unpaired X chromosome,-i.e. 18+x. This unpaired X chromosome shows heteropycnotic properties, i.e. capacity to get stained heavily or lightly in comparison

with other chromosomes. During the first few stages of Prophase I, it is positively heteropycnotic and thus helps in identification.

+ve heteropycnosis - densely stained body.
-ve hetropycnosis - not so.

Prophase I

It has 5 substages, during which homologous chromosomes pair and exchange genetic matter.

(a) Leptotene: (i) thin chromosomes, delicately laid out, within the nuclear membrane.

(ii) chromisomes appear beaded because of tiny bead-like structures-the chromomeres.

(iii) very often, these chromosomes have a definite polarisation, and form loops whose ends are attached to the nuclear envelope. This peculiar arrangement is called the "bouquet" and the point of attachment represents the area of the centrioles.

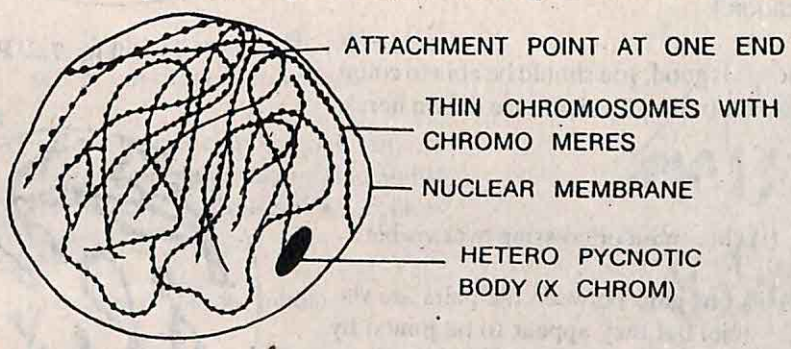


Fig. 7.4. Leptotene

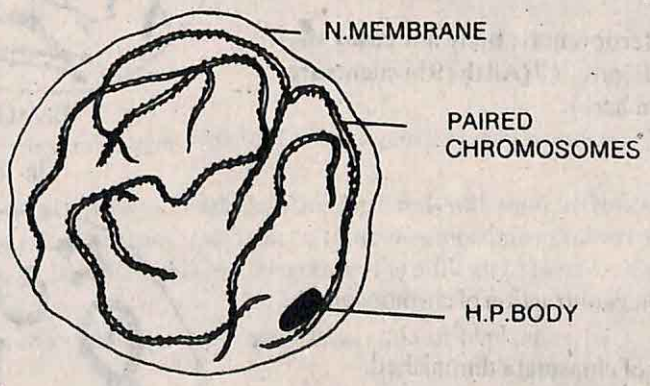


Fig. 7.5 .Zygotene

(b) *Zygotene:* (i) homologous chromosomes stains start pairing, so chromosomes appear slightly thicker.

(ii) chromomeres are still present.

(iii) hetero pycnotic body present.

(iv) because of pairing there is more space within the nuclear area.

(**Note :** one pair = single bivalent)

(c) Pachytene : (i) pairing completed and chromosomes contract longitudinally thus appearing difinitely thicker.

(ii) nucleus appears to contain half the number of chromosomes.

(iii) heteropycnotic body visible.

(iv) often, knob, like thickenings visible.

(**Note :** Each bivalent is made up of 4 chromatid because of duplication.)

If the microscope is good, you should be able to count 9 bivalents. Figure. 7.6 (only 4 bivalents are drawn here).

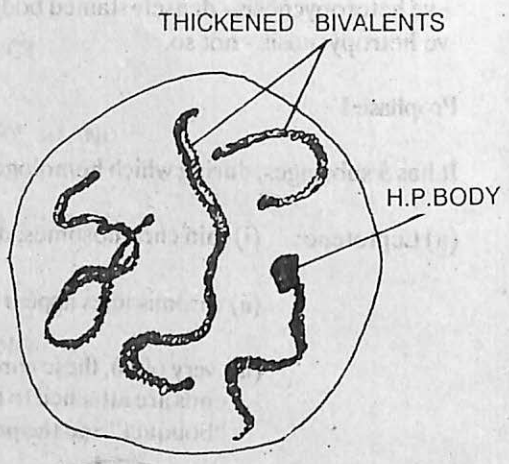


Fig. 7.6. Pachytene

(d) Diplotene : (i) chiasmata or crossing over visible.

(ii) tiny gaps between the pairs are visible, but they appear to be joined by their points of chisma formation.

(iii) heteropycnotic body not easily visible. Figure. 7.7 (All the 9 bivalents are drawn here).

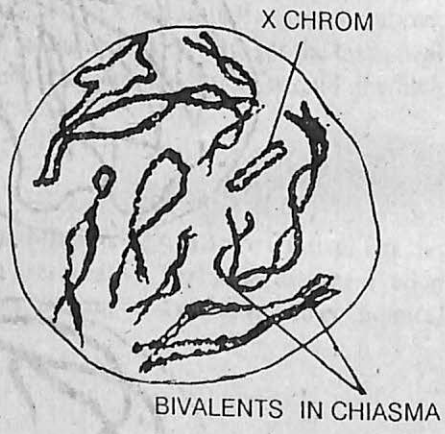


Fig. 7.7. Diplotene

(e) Diakinesis : (i) further contraction of chromosomes.

(ii) no. of chiasmata diminished.

(iii) due to separation and terminalisation of chiasmata, the bivalents are held together only by their ends.

(iv) bivalents during separation, also undergo rotation and assume characteristic shapes.

(v) nuclear membrane not so visible.

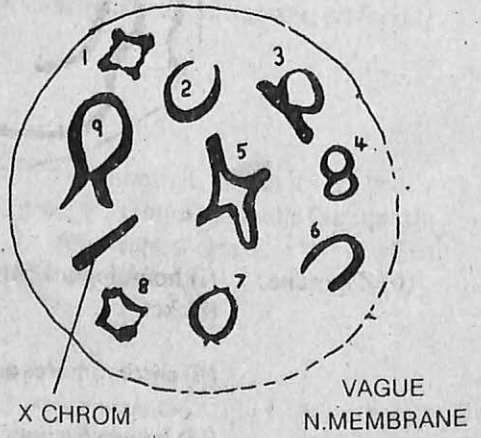


Fig. 7.8. Diakinesis

(vi) H.P. body or the X chromosome not so visible.

9 Bivalents + X Chrom.

Metaphase I: (i) characteristically shaped bivalents of diplotene are seen on the equatorial plane.

(ii) nuclear. membrane disappears.

(iii) if a polar view is observed, then these bivalents will be seen in a circular fashion (Fig. 7.9).

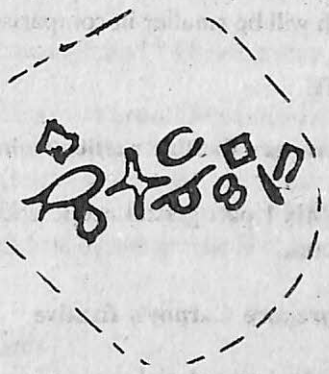


Fig. 7.9. Metaphase I

Anaphase I: (i) bivalents separated. Therefore, two sets of univalents can be seen.

(ii) if the microscope is good you can count 9 univalents on one side and 10 on the other (because of X Chromosome).

(iii) typical anaphase spindle.

(iv) chromosomes short and thick (Fig. 7.10).

Fig. 7.10 Anaphase I

Telophase I : (i) two small nuclei with 9 or 10 univalent short chromosmes.

(ii) telophase I and Prophase II are small cells with short and thick chromosomes and no other particular diagnostic features. If the chromosome number can be counted, then they can be definitely identified. Prophase II is difficult to locate, as it is of a very short duration.

Metaphase II : (i) univalents arrange themselves at equatorial plane.

(ii) note the shape of chromosomes which are more like mitotic chromosomes. (Can distinguish easily from metaphase I).

(iii) chromosomes will be either 9 or 10 in nember.

Anaphase II : (i) chromosomes thinner.

(ii) poles will have equal number, i.e. either 9+9 or 10+10.

Telophase II : Small cells with haploid chromosome number thinner chromosomes. If it is a late telophase, then it will look like an interphase nucleus, and difficult to identify as telophase -except for the size of nucleus, which will be smaller in comparison with a typical interphase nucleus.

NOTE :

To prepare fixative acetic alcohol

Mix 1 part glacial acetic acid with 3 parts absolute alcohol. Keep material in fixative for at least 15 minutes.

To prepare Carnoy's fixative

Mix 1 part glacial acetic acid with. 3 parts chloroform and 6 parts 100% alcohol. Fix for 3 hours, remove and wash in distilled water then preserve in 70% alcohol.

To prepare acetocarmine stain

Glacial acetic acid - 45 ml.
Distilled water - 55 ml.
Carmine powder in excess, i.e. 1 gm.

Dissolve carmine powder in acetic acid. Mix with water and boil vigorously for more than 30 min. and then cool. If needed, dilute with 45% acetic acid.

QUESTIONS

Q.1. At what magnification should the slide of meiosis be observed ?

Ans. In high power, i.e. at 400 x magnification.

Q.2. What is the event that occurs throughout prophase regardless of whether it is meiosis or mitosis ?

Ans. Chromatin network disorganises, and clear thread like chromosomes are visible. Nuclear area enlarges, nuclear membrane disappears.

Q.3. In the two nuclear divisions of meiosis, during which one, reduction as well as variation are achieved?

Ans. During the first nuclear division.

Q.4. What is a bivalent ? What is the significance of its formation ?

Ans. A bivalent is a pair of chromosomes (homologous) and is a tetrad structure as each chromosome has two chromatids. Their formation is significant since pairing of material and paternal chromosomes finally lead to crossing over or exchange of genetic matter, which in turn results in variation among gametes.

Q.5. What are chromomeres ?

Ans. Chromomeres are thickenings along the length of chromosomes. These occur during condensation of chromosomes which is not uniform throughout the length of the chromosome.

Q.6. What is the difference between mitotic metaphase and meiotic metaphase I ? How are they similar?

Ans. They are similar in the feature that during metaphase all chromosomes are at the equitorial region. They are different as, in mitotic metaphase single chromosomes (duplicated with 2 chromatids) line up on the equator, whereas in meiotic metaphase I, bivalents (tetrad structures) are at the equator. Each centromere in the mitotic metaphase is held by two spindle fibres - one from either pole. In meiotic metaphase I, the two centromeres in the bivalent are each held by one spindle fibre from either pole.

Q.7. Chromosomes appear short and thick from which stage onwards ?

Ans. Maximum condensation is visible during diakinesis and metaphase I.

Q.8. When you compare diplotene and diakinesis, the number of chiasma points appear less. Why?

Ans. This is because the chiasma or the crossed over chrmosomal parts shift towards the end (having completed exchange) or terminalise and finally slip off.

Q.9. What is the difference between telophase II as seen in anther smear and in grass hopper testes ?

Ans. In the anther smear, spore tetrads are formed, all the four haploid cells stay together and hence are easily visible. This is not so in the other material, as products of telophase get scattered in the slide preparation .

Q.10 In which stages can you see the H.P. body clearly ?

Ans. The heteropyenotic body (H.P.) can be clearly seen in leptotene and zygotene stages.

CORE EXPERIMENT 8

Aim : *Study of variation in leaf size, pod length, seed size and seed number.*

Variations

Variations are dissimilarities or diversities exhibited by organisms. Variations occur at all levels, i.e. among members of the same species, among different species, genera and so on. Variations are observed among children of the same family, among flowers, fruits and leaves of the same plant.

Variations are caused because genes interact with environmental factors and produce specific phenotypic expressions. Variations are also caused because genes undergo changes (mutation) or chromosomes undergo changes in their number or size.

Why do we study variations ?

1. It is interesting to learn about diversities in nature.

2. Nature of variation helps us to know whether it is a mutational variation or is simply caused by environmental factors.

3. In a given trait, we can observe the range of variation by statistical interpretation such as by histograms.

Following is a simple method of studying variation in leaf size and some features of pods.

(A) **Leaf size variation**

Material Required : A healthy potted plant with abundant leaves, eg - chrysanthemum, Money plant, Dahlia etc., graph paper (mm squares) and pencil.

Procedure : Find out the smallest leaf and the largest leaf. Measure them from base to tip with a scale. Once you know the range (say 3 cm & 7 cm) decide upon 3 sizes of leaves. For example.

3 - 4 cm - Small leaves.
4 - 5 cm - Medium leaves.
More than 5 cm - Large leaves.

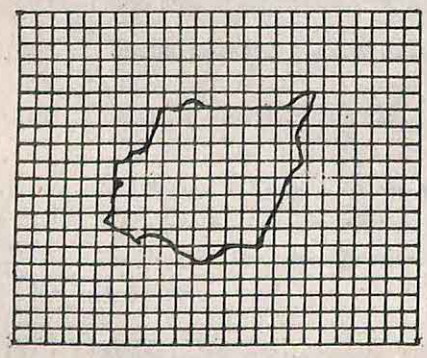


Fig. 8.1

With the help of graph paper measure area of at least 50 leaves at random. For this, place graph paper beneath the leaf, draw an outline of the leaf. Count squares and calculate area in sq mm, Ignore squares which are less than half covered. Count squares which are half or more than half covered. Take the measurement of one suare before you start counting them. Measure the length of each leaf with a scale. Write both data in the same unit, i.e. mm or cm. Record observations as suggested below.

Leaf serial	Length	Category	Total no of. small	medium	large	Area
1		Small	10		10	X
2		Large				Y
3		Medium		30		Z
↓ 50						

Table 8.1

Discussion : Comment upon whether there is predominance of any one category or all 3 are randomly present. The average area of small, medium and large leaves could be found out. The range of variation in each category can be discussed. Interpretation of the data can be plotted in terms of histograms. For this, prepare suitable frequency distribution for lengths and areas and plot them against number of leaves.

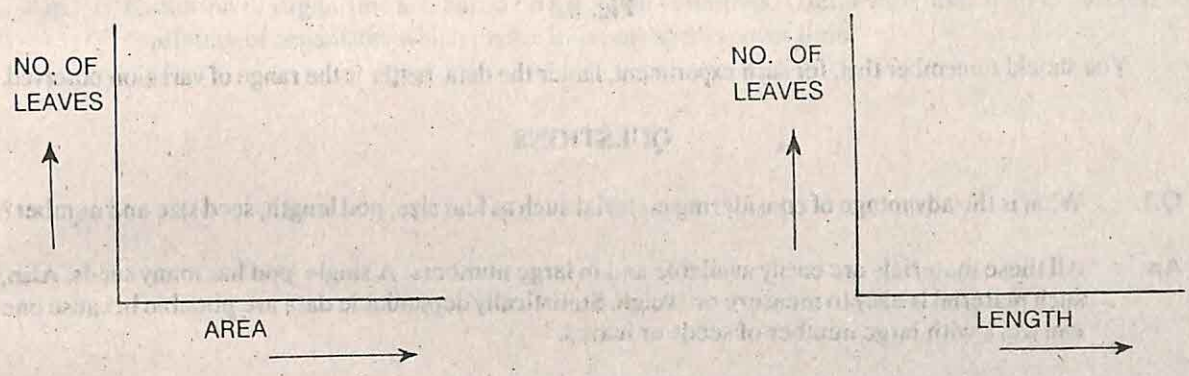


Fig. 8.2

(B) **Pod Length, number and size of seeds.**

Material Required : Pea pods or beans, thread, scale.

Procedure : Each student (or groups of two) take at least 50 pods or beans. Measure the following.
(a) Length of pod with scale.
(b) Circumferance of each seed with a thread.

Take the thread round the middle of the seed, & then measure this piece of thread by placing on the scale. Count number of seeds in each pod. Tabulate your observations as follows :-

Serial no.	Length of pod	Number of seed in each pod	Circumferance of seed
1			
2			
3			
↓ 50			

Table 8.2

NB : You could also add seed colour (green, palegreen, yellow) and seed texture (smooth or wrinkled).

Discussion : Comment upon average length, number and seed size. From the data, see if there is any predominance of any particular length, number or seed size. Interpret the result interms of histograms, by making suitable frequency distributions. For example. :-

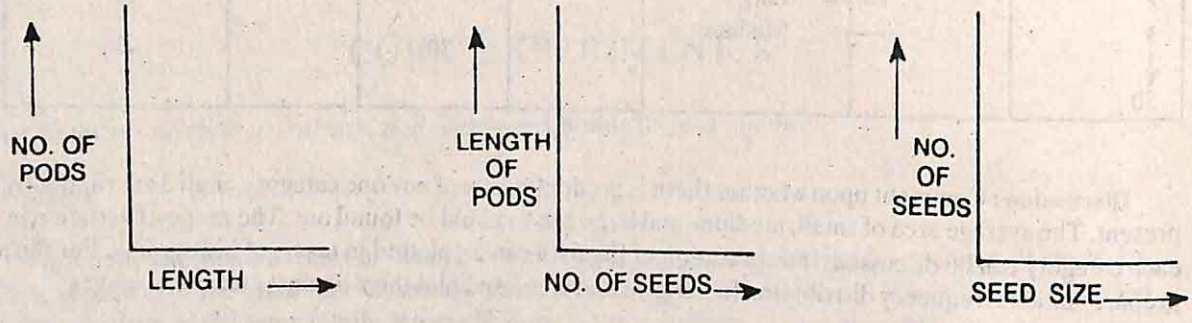


Fig. 8.3

You should remember that, for such experiment, larger the data, better is the range of variation observed.

QUESTIONS

Q.1. What is the advantage of considering material such as leaf size, pod length, seed size and number?

Ans. All these materials are easily available and in large numbers. A single pod has many seeds. Also, such material is easy to measure or weigh. Statistically dependable data are possible because one can work with large number of seeds or leaves.

Q.2. How is the graph paper useful in measuring area of leaf ?

Ans. The graph paper is made up of small squares with known area, therefore simply counting them helps in knowing the area of the leaf. The normal procedure of multiplying length and breadth to get area is difficult to apply as, leaves have varying width along their length.

Q.3. Which kind of variations - environmental, or genetic are easy to observe and study ?

Ans. Environmental variations are easy to observe and study. Changes in light, temperature, soil condition, water etc bring about quick and noticeable changes resulting out of interaction of the genes with these environmental factors.

Q.4. What knowledge will you gain by plotting frequency distribution graphs ?

Ans. One can know about the number of individual leaves/seeds, etc. in a given category of variation, and hence the predominance of a type or whether all variation types are of equal frequency etc.

Q.5. Why do we not consider animal specimens for such study ?

Ans. Animals offer certain problems of housing, feeding, in addition to the fact that large number of one type of animal are often not possible to obtain. They are mobile and difficult to handle under ordinary lab conditions.

Q.6. Considering leaf, seeds, pods, where do you expect more range of variation - why ?

Ans. Normally leaves will show size variation more obviously than others, as all plants have leaves in different phases of growth. Pods and seeds will not show so much variation (of the same plant) if they are fully mature as no further growth is evident.

Q.7. Suggest some possible variations of this experiment to make it more elaborate and interesting.

Ans. For leaf size - different varieties of a given plant (e.g. - Rose) could be considered. Leaf size variation of the same plant in different seasons could be considered.
For seed size, pod length - similar modification as above can be followed.
Other features such as flower colour, size, margin, apex or base shapes of leaves could be considered.

Q.8. How are variations significant in nature ?

Ans. Evolution of organisms are based on heritable variations. Useful variations help in survival and continuity of organisms which evolve into new species over time.

CORE EXPERIMENT 9

Aim : *(a) To study nitrogen fixation in blue green algae by protein test.*

Material Required : Blue green algae. (You may collect from drains, ditches, mortar, sand water logged fields with blue green growth on the soil surface, damp flower, pots outer surface.) pestle and wortons , sand, distilled water, conc. nitric acid, conc. liquid amonia or conc. NaOH, Millon's reagent, sprit lamp, test tubes, filter paper, funnel, iron stand.

Method - (Procedure)

Collect the samples of blue green algae . Take about spoonful of one of the sample and mix with equal quantity of sand. Place the mixture in a mortar and grind till a paste is formed. Add about 5 ml. of distilled water and filter. Collect the filtrate.

(a) Pour about 3 ml. of the filtrate in a fresh test tube. Mix 1 ml. of conc. nitric acid. Yellow colour appears. Heat to boil and cool. Add equal quantity of liquid ammonia or conc. sodium hydroxide and see if there is any change in the colour of the precipitate.

(b) Take in another fresh test tube about 3 ml. of the filtrate and mix about 2 - 3 drops of Millon's reagent. Note if there is any precipitate is formed. Heat the test tube and cool. See if there is any change in the colour of the precipitate.

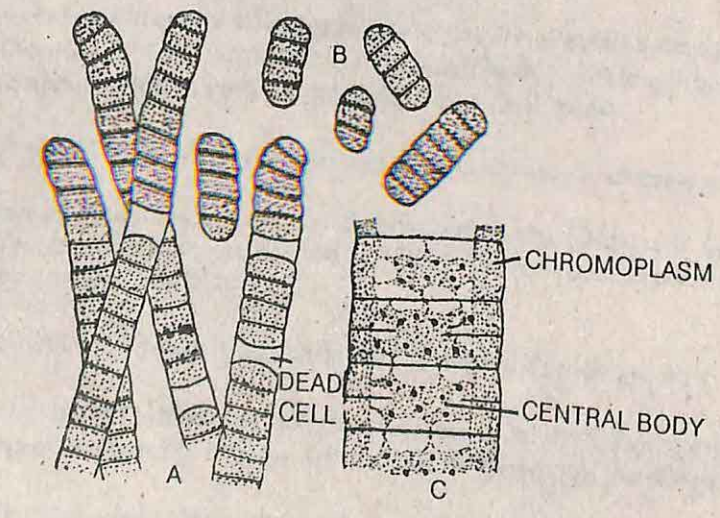


Fig. 9.1 Blue green algae Oscillatona

Observation :

(a) In the test tube the colour of the filtrate changes into yellow. Later, on heating it changes to orange. This is a positive test for protein.

(b) In the second test tube proteins are precipitated and take whole yellow colour. On heating the colour changes into red. This is a confirmatory test of proteins.

Result : The positive test of proteins are indicated.

Discussion : Blue green algae (Cynobacteria) are autotrophic in nature because of the presence of chlorophyll and phycocyanin pigments. These pigments help in the fixation of atmospheric nitrogen into proteins. Thus the protein test carried out with the filtrate confirms the property of blue green algae.

Precautions :
1. Handle the chemicals carefully.
2. Collect fresh blue green algae for fresh experiment.
3. You can culture blue green algae in the Lab also.

Aim : *(b) To study root nodules in a leguminous plant like pea / bean / gram.*

Material Required : Sample of roots of leguminous plants with root nodules intact, methylene blue, microscope, glass slide, glass coverslip, blade, Thread, sprit lamp, dil. NaOH, $CuSO_4$. Mortar & pastle, distilled water.

Procedure :

(i) Wash the root system thoroughly under tap water and remove the root nodules. Measure the total length of the root system by using a thread.

Count the nodules that have been separated and measure their diameter. Record your observation in a table.

(ii) To prove that atmospheric nitrogen is fixed in root nodules. you can perform a chemical test. For this test, you can make a mince of few root nodules (20) and 2 ml. of distilled water. Collect the fluid part from the mince in a test tube (about 2 ml). Add about 2 ml. NaOH and 2-3 drops of dil. $CuSO_4$ solution and shake the tube. Look for the appearance of colour. Heat and note the change in the colour into violet.

(iii) For microscopic observation of the bacterial cells prepare a slide. Place a drop of the minced cap on a slide and spread evenly. Allow to dry. Add a drop of methylene blue and leave for a minute. Warm over a spirit lamp. Remove extra stain by holding slide on a watch glass and sprinkle distilled water. The blue stains will be retained by the bacterial cells and rest of the slide will be clear. Now add a drop of diluted glycerine and place a coverslip. Focus under the low power and then high power to locate Rhozobium cells. If the stain is proper, you will see rod shaped cells. The incepient nucleus may not be distinctly visible.

Observation : Record your observations of the experiment in the following table

Name of the plant system	No. of nodules	Range of diameter	Total length of thread
Pea Bean Gram			

(i) In the experiment positive test of amino acid is confirmed.

(ii) Rod shaped bacterial cells are seen.

Discussion : The root nodules are produced by the roots, when bacterial cells invade the root hair and reach into cortical tissue. Here the cortical cells harbour Rhozobium colonies and enlarge in the size.

Thus, the root cells are not damaged due to ammonia. Later, these amino acids are utilised in the preparation of proteins by the legume plants. The Rhozobium cells in turn can make use of the place and other basic needs like water, carbohydrates and minerals. Thus, there is a perfect establishment of symbiosis.

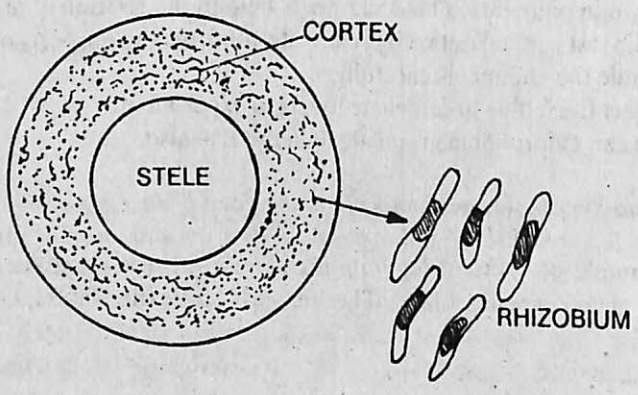


Fig. 9.2. T.S. Legume root showing Rhozobium in cortex

QUESTIONS

Q.1. Why are blue green algae and bacteria included in the same kingdom ?

Ans. Both of them are prokaryotes.

Q.2. To what kingdom do the above said organisms belong ?

Ans. Kingdom Monera.

Q.3. Give some of the characteristics of Monera.

Ans. The organisms do not possess well defined nucleus. They lack nuclear membrane. The cell organelles like mitochondria, chloroplast, endoplasmic reticulam, etc. are not found.

Q.4. Name the pigments found in blue green algae.

Ans. Phycocyanin., Chlorophyll

Q.5. Name the bacteria associated with root nodules.

Ans. Rhizobium.

Q.6. Name any other free living bacteria that fix atmospheric nitrogen.

Ans. Nitrosomonas.

Q.7. Why is it necessary for Rhizobium to live in the leguminous roots ?

Ans. Due to physio-chemical reactions and is called symbiotic relationship.

Fig. 9.3 Asymbiont-Rootmodule

CORE EXPERIMENT 10

Aim : *Study of two plant diseases of the area, identification of causal organisms, alternate hosts and carriers.*

Material Required : Microscope, needles, blade, slides and coverslips and Fresh and preserved materials of any two of the following :-

1. Wheat or barley rust
2. Sugarcane smut
3. Red rot of sugar cane
5. Late blight of potato
6. Canker of citrus
7. Blast of rice
8. Green ear diseese of bajra
9. Ergot of bajra
10. Loose smut of wheat
11. Smut of bajra
12. Downy mildew of cucurbits

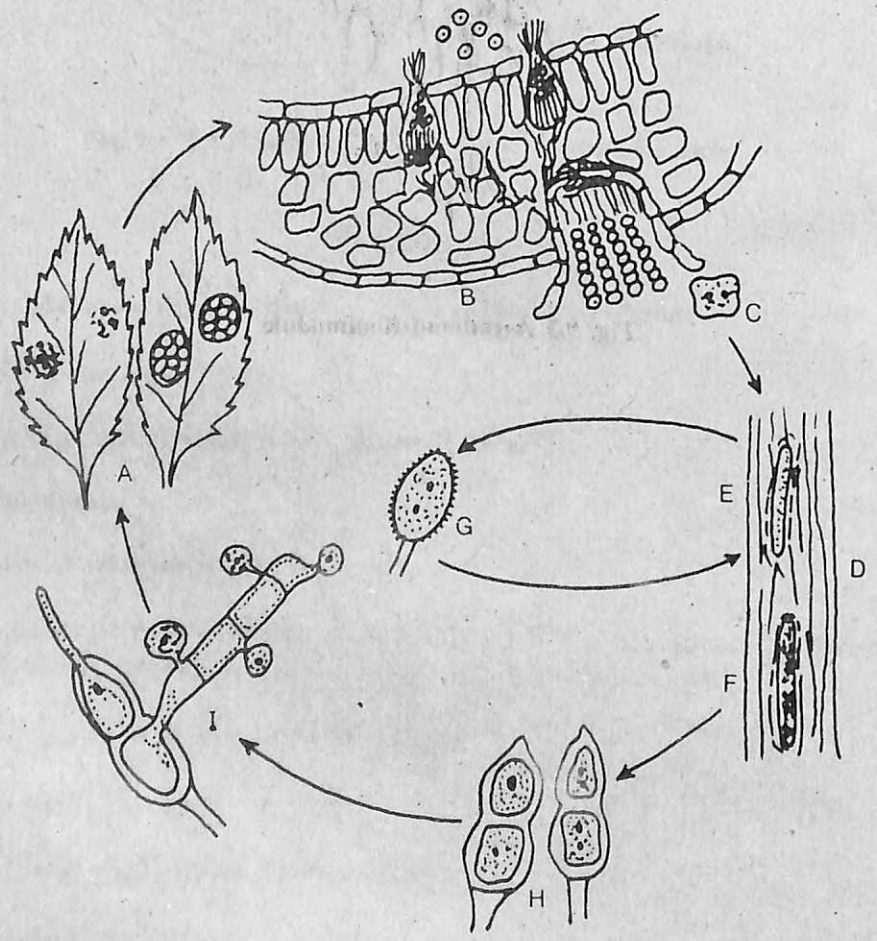


Fig. 10.1. L.H. of Puccinia graminis tritici

A. *Berberis leaf showing aecidial cups*
B. *V.S.Leaf of A.*
C. *Aecidiospore*
D. *Wheat leaf showing pustules*
E. *Urediospore pustule*
F. *Teliospore pustule*
G. *Urediospore*
H. *Teliospore*
I. *Basidium*

Procedure :

1. Wheat Rust - Causal organism - Puccinia graminis. Collect the diseased portions of stem and leaves of wheat. Study the change in the appearance and colour of the leaf and stem. Note the presence of pustules scattered in streaks on the host. Draw a sketch. With the help of a needle scrape out a pustule and mount on the slide. Study the slide under a microscope. Draw the sketch of the spores you find in the microscopic field. Cut a thin transverse section of the leaf passing through the region where the parasite is growing. The origin of spores can be studied here. These are urediospores.

From the stem cut a transverse section and study the parasite. You will see difference in the spores. Note the colour cell wall, and number of nuclei in spores. These are teliospores.

Observation : Two different types of spores can be seen in two different types of pustules. The pathogen completes its cycle on two hosts. Wheat is primary host. On Berberis leaf Pycnidiospores and aecidiospores are seen. Note the morphology of aecidiospore by mounting on a clean slide.

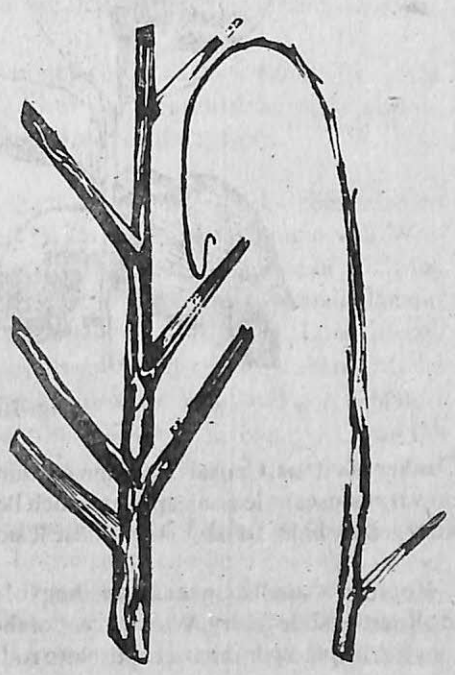


Fig. 10.2. Sugarcane smut

The aecidiospores can attack on fresh crop of wheat in the next season.

This disease is air-borne.

2. **Sugarcane smut - 'Ustilago scitaminea'.** This disease is seen as a coiled whip-like structure coming out of the apical end of the plant. The coiled structure appears to be covered by a silvery covering but it explodes black ripe spores. Take out the spores and study under the microscope. You can locate few such lateral branches from lower nodes of a sugarcane. Draw the diagrams.

3. Late blight of potato causal organism Phytophthera infestens. The disease is seen during early January when damp weather prevails. Small purplish lesions appear from the tip and margin spreading inwards. These lesions show whitish grey mildewy growth on the lower side of the leaf. Collect infected leaves and study the lesions carefully under a dissecting microscope. Some of the aerial growth will be seen.

Remove a small piece of infected leaf and place on a clean dry slide. Add one drop of cotton blue and leave the material. Add a drop of lactophenol and cover with coverslip. Study under microscope. Note the

presence of coenocytic mycelium and sporongia on branched sporoangiphores. Draw the diagrams.

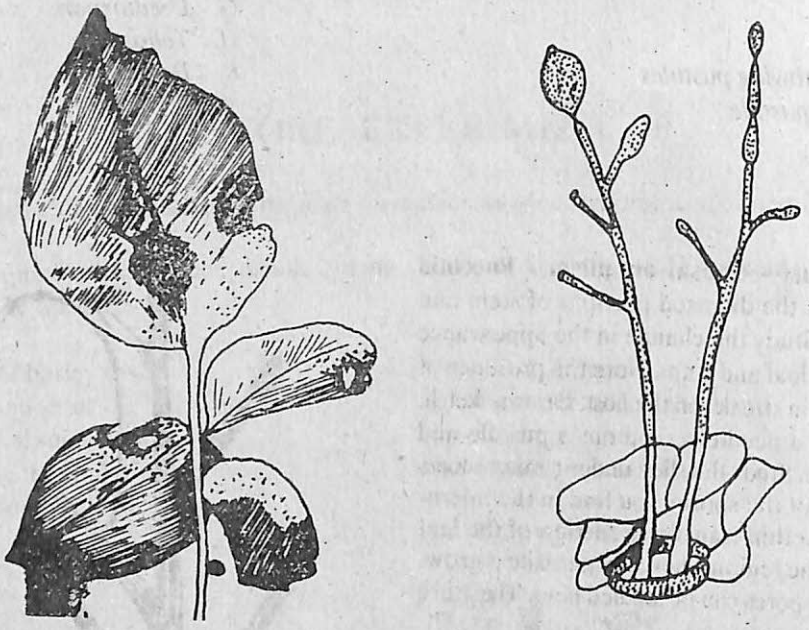


Fig. 10.3. Late blight of potato

4. Canker of citrus. Causal organism anthomonas citri. The disease occurs on twigs, leaves and fruits. Small yellow transluscent lesions appear which become brown at later stage. These lesions are surrounded by a watery yellow halo. In later stage these lesions fuse together and form a Canker.

Remove a small Canker by the help of a needle and spread on a dry slide. Add a drop of crystal violet and allow the slide to dry. Wash excess of the stain with water and add alcoholic potassium iodide and study under the high power microscope. Note rod-shaped bacteria.

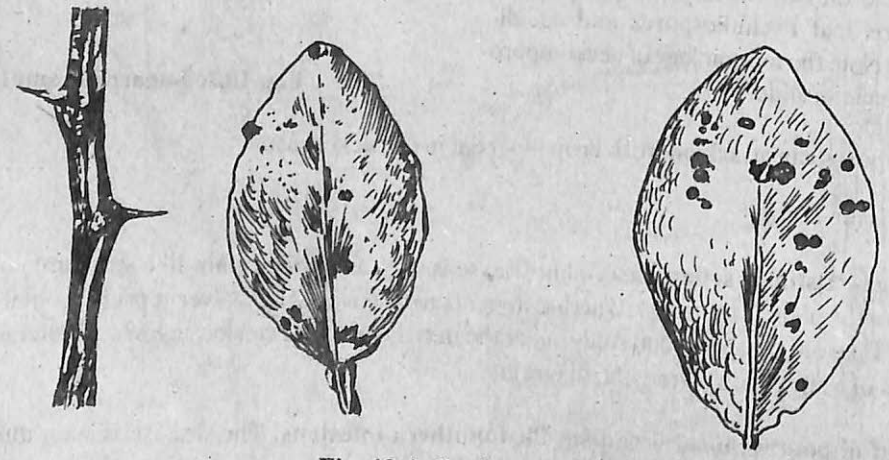


Fig. 10.4. Canker of citrus

5. Smut of Bajra. Smut of bajra is very common in many parts of India. The infection is visible on scattered grains in the ear. Majority of grains escape damage. The spores of the fungus causing smut of bajra are held together in compact balls which remain perisitent even in water. These balls appear stony green in early stages and become dark black on maturity.

In an infected ear look for the difference in size of healthy and infected grains. With the help of a needle open one of the infected balls. What do you find ? Mount a small portion of powdery mass on a clean slide with a drop of water. Study under the microscope. Note the individual spores - their shape, size colour, texture of the wall whether smooth or rough. Locate the spores which have germinated inside the balls. What do you notice. Is the promycelium single celled or many celled. In your opinion what is the use of spore balls ? Do they help in dissemination of the pathogen or perennation during the rest of the period ?

6. Ergot of bajra. Causal organism - Claviceps microcephala. This disease is common in northern and southern India. The damage caused by the disease depends upon the weather at the time of ear formation.

The disease is conspicuous as small droplets of pinkish or light honey-coloured fluid, exuding from the spikelets. Later these droplets become darker and fuse together and cover a larger area of the inflorescence. In later stages, small dark brown sclerotia can be seen projecting from between the glumes.

Fig. 10.5. Ergot of bajra

To study the diseased plants - pick a contaminated bajra spike and locate darker grains hanging out. With the help of a needle pierce through one of sclerotia. Take out the black powdery mass with small filamentous material and mount on a clean slide. Look through the microscope. Do you find details of filaments ? Is the mycelium sepatate or coenocytic and non septate ? Note the colour and shape of the conidia. Count the number of nuclei in each segment.

7. Loose smut of wheat. Causal organism - Ustilago tritici. Loose smut can be a very destructive disease of wheat in susceptible varieties. The disease shows its appearance at the time of ear formation. Almost every head of the affected plant is converted into a black mass of spores and no grains are formed. These spores developed in the young spikelet, covered by a delicate silvery membrane which usually bursts leaving olive brown to black powdery mass in place of normal spikelets, of which only the end of awns are not affected. The spores easily separate from each other and are blown with the wind leaving behind a bare rachis. To study the fungus pick up an affected ear and study the symptoms - do you find any grain developed? Shake the spikelet. What happens ? Pick up some of black powdery mass and mount on a slide. Study undera microscope. There are large number of spores - note the colour, shape, size of the spores and number of walls. Try to locate spores in which germ tubes have been formed. Draw a labelled diagram of the host with symptoms and the spores.

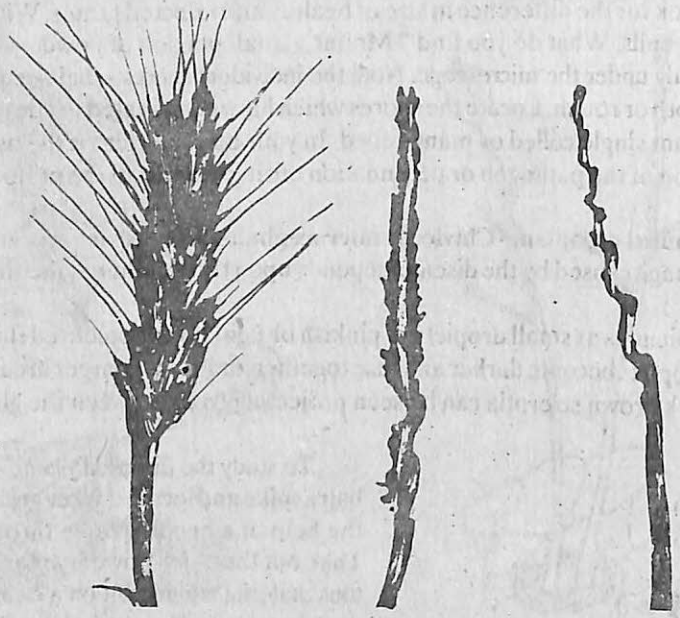


Fig. 10.6. Loose smut of wheat

8. Downy mildew of cururbits. Causal organism - Pseudopemonospora cubensis. The disease is fairly common in Northern India during the later part of the rainy season. The leaves show angular spots - purplish growth appears on the lower side and pale yellow on the upper side. The affected region can be picked up by forceps and stained with cotton blue and then studied under microscope. Look for mycelium. Do you find it coencytic or septate? How is it spreading ? Intercellular or intra-cellular. Locate ovate haustoria. Look through the stomatal region. Sporangiophores can be seen - are they branched or single filamentous ? Study the sporangia size, shape, origin, colour, thin walled or thick walled.

Fig. 10.7 Downy mildew of Bajra

9. Downy mildew of bajra. (Green ear disease of Bajra.) Causal organism - Sclerospora graminicola. Downy mildew is a common disease of bajra and sometimes results in heavy losses due to its disturbing effects on the ear. The disease shows its presence in two stage - the downy mildew stages which is predominant on the leaves and the green ear stage affecting the ears. In India green ear stage is more prevalent.

The affected plants show stunted growth. Leaves trun yellow in streakes on the upper side and the lower side is covered by a fine downy growth of the fungus. Sometime the floral buds develop into bunchy appearance. Examine a small portion of the leaf under microscope and study the nature of mycelium, sporangia and sporangiophores.

The principal symptoms are produced in the inflorescence, which is deformed and characterised by the transformation of floral organs into twisted leafy structures. This gives the appearance of green leafy mass, hence called "green ear".

Select one of the diseased leaves and look for brown, smooth walled, irregularly shaped to spherical structures. These are Oospores, which perennate in the soil along with old diseased leaves. They need some resting period or weathering before germination. What do you suggest - is the disease soil-borne systemic or air borne.

10. Red rot of sugarcane. Causal organism - Colletotrichum falcatum. Red rot is one of the most common and serious diseases of sugarcane in India. The disease attacks standing canes. The symptoms are shown on leaf and stem both. The leaves show red patches with ash coloured center in the mid rib region. With the help of a needle pierce through one of the lesions and study under a microscope with a drop of water.

Look for the infection - is it colourless or deep coloured. Sepatate or not. Study the conidiophores and conidia. Study the shape (sickle-shaped), size and colour. In slightly older lesions you may find chlamydospores thick walled, dark.

In the canes the pith region becomes reddened; small cavities filled with mycelium can be seen and juice gives an alcoholic odour, scattered in the mycelium look for minute, dark velvety dot like structures. Study them under the microscope. These are conidia.

11. Blast of rice. Causal organism - Pyricularia oryzae. Rice blast or "rotten leaf" is chiefly a foliage disease, the symptoms are found on leaf sheaths, rachis, joints of the culm and even the glumes. The disease is serious in humid areas.

Fig. 10.10. Midrib infection caused by C.falcatum.

Fig. 10.9. Dry, shievelled cane due to attack of C. falcatum

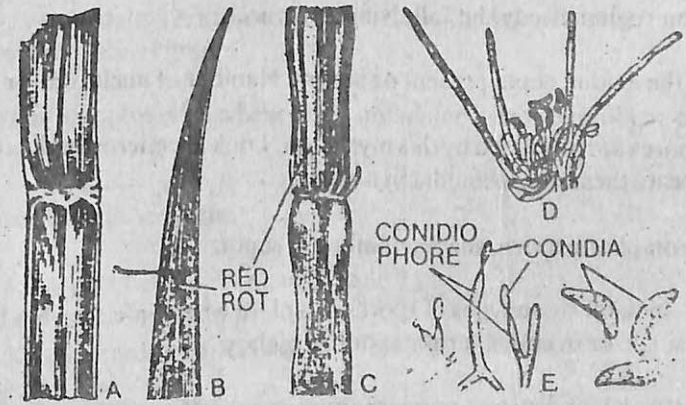


Fig. 10.8. Red rot of Sugarcane A-C. Disease symptoms A and C. On stem. B. On leaf. D. An acervulus. E. Conidiophores

Fig. 10.11. Blast of Rice

In the leaves small water-soaked greyish - brown lesions or black rings are formed on the rachis of maturing panicles. In the culm region neck shrivels and turns into bluish patch with a grey fluffy mycelium. The neck region becomes weak and the inflorescence tends to fall.

To study the fungus, prepare a slide by removing the infected portion from the leaf or culm region. Tease the material and stain with cotton blue. Under the microscope study the following :

Mycelium - is the mycelium septate or not, how many nuclei - one or many, branched or unbranched, colour ?

Condiophores - are they simple or branched ? Sepatate or of one segment ?

Conidia - are they produced terminally or laterally. Shape (ovate or spherical), septate or single celled, uninucleate or multinucleate ?

12. Wilt of pigeon pea (Arhar - cajanus indicus). Causal organism - Fusarium oxysporum var-udum. The arhar plant is susceptible to wilt attack throughout its development. Main symptom is wilting of leaves as if water shortage is in the field, followed by yellowing, withering and drying of leaves and may be the entire plant. Examination of main root and stem shows entire portion as blackened in the xylem region mainly. Partial wilting is also common where stem on one side is blackened and the rest of the plant remains healthy. The formation of seed is restricted.

To study the pathogen, spilt open the affected root and stem lengthwise. Do you find discoloured conducting tissue ? Cut out thin transverse section of stem and root. Stain with cotton blue and look for the mycelium in the xylem region. Study the following structures :-

Mycelium - note the colour, septa present or absent. Number of nuclei one or many.

Three types of spores are produced by this mycelium. Look for microconidia - comparative size, shape, cellular structure. Locate these microconidia in a cluster.

Macroconidia - comparative size, shape, number of septa.

Chlamydospore - look for these types of spores in a place where infection has been old enough. Locate round, thick walled, single or in chain, terminal or intercalary.

N.B. - Select any two plant diseases prevalent in your area. If possible make field trips for on the spot studies. The expected loss to the crop can be estimated by the extent of damage caused to the crop.

QUESTIONS

Q.1. Why is the name given "Rust" to wheat leaf infected by Puccinia graminis tritici ?

Ans. Brown coloured lesions developed on the leaf between the veins give a look of Rust of iron.

Q.2. How many types of spores are found in the life history of the causal organism ?

Ans. Five types-Basidiospore, Pycnidiospore, Aecidiospore, Urediospore and Teliospore.

Q.3. Name the alternate host.

Ans. Barberries vulgaris.

Q.4. How are Urediospore different from Teliospore ?

Ans. Urediospores are single celled, binucleate with two thin walls having one or two germ pores. Teliospores are two celled, single nucleated, with thick wall having one germ pore in each cell on alternate side and a well defined stalk.

Q.5. In India Barberis vulgaris is not considered to be the alternate host of wheat rust. How does the rust reccur in the next year ?

Ans. This has been proved experimentally that the alternate host is not seen in India. However the reccurrence of the disease is possible by the urediospore that travel along the wind from one region to another. Wheat is been cultivated the year around in our country, thus the urediospores from the fresh crop can be disseminated from the fields.

Q.6. List the symptoms of the disease.

Ans. Brown pustules of urediosori appear on wheat leaves, which may gradually spread into the leaf sheath and stem. The height of the plant is retarded and in later stage the grains produced are shrivelled.

Q.7. How do you differentiate between rust and smut of wheat ?

Ans. In the rust, leaves and stem are affected. In the smut, the inflorescence in affected. A black powdery soot is present in place of grains.

Q.8. Give important symptoms of wheat rust, sugarcane smut, late blight of potato and red rot of sugarcane.

Ans. Refer the text of the experiment.

Q.9. Why is the name given as red rot of sugarcane ?

Ans. The stem setts show red streaks along with foul alcoholic smell.

Q.10. Name the causal organism of red rot of sugarcane.

Ans. Colletotrichum falcatum.

Q.11. What are the symptoms of wilt, canker and downy mildew ?

Ans. Refer the text of the experiment.

Q.12. Suggest the method of control of the diseases.

Ans. Prophylactic, Theraupticand Immunization.

Q.13. What is biological control ?

Ans. It is a prophylactic method of disease control, with the help of another living organism. The roots of certain plants secrete toxic substance that can check the growth of pathogens. Some microorganisms have antagonistic effect on the growth of some soil microorganisms.

CORE EXPERIMENT 11

Aim : *Study of stages of life cycle of Slik Moth or Butterfly.*

Fresh Material, Sources, Collection, Preservation, Silk Moth.

Eggs of silk moth appear yellowish and are nearly spherical. They are laid in several hundreds and look like patches or tiny clusters on leaves. Both eggs and larvae can be easily collected from Mulberry plant leaves.

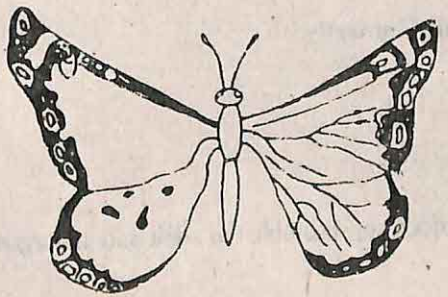
Commonly available examples of butterfly are *Danais* and *Papillion* (Figs 11.1 & 11.2).

Insect eggs can be preserved in 70% alcohol. Insect larvae (caterpillars) can be killed and preserved in 80-90% alcohol. Life cycle stages can be very easily watched in living material. Eggs and or larvae can be collected and fed on their natural food, e.g., mulberry leaves. These can be kept in card board box (shoe box), covered with celllophane paper (to be able to see) and tiny holes made in the box for aeration. In fact catterpillars can be collected from various sources like cabbage leaves, cauliflower, brinjal, bhindi etc. Each can be easily maintained with their respective food types and development can be watched. Well defined stages can be picked up and preserved in labelled containers.

Life cycle of silk moth and cabbage white butterfly is shown in figure 11.3 and 11.4 respectively. The stages are very similar in most moths and butterflies. Students can make observations on comparative basis regarding minor differences, and differences in time periods.

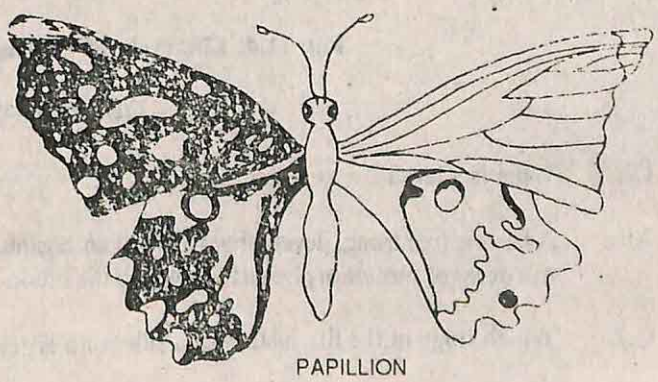
Larvae feed on mulberry leaves and do not roam about, have a length of 45-55 mm. Larval period lasts for 45 days and then pupates to form a cocoon. Cocoon - is white, thick and oval. Adult emerges from the cocoon in 12-16 days.

Cabbage white butterfly - feeds on cabbage leaf. At rest wings are folded in vertical position.



DANAIS

Fig. 11.1



PAPILLION

Fig. 11.2

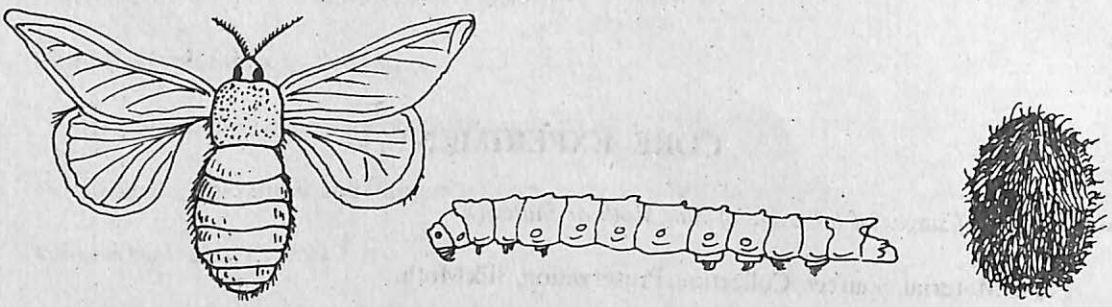


Fig. 11.3

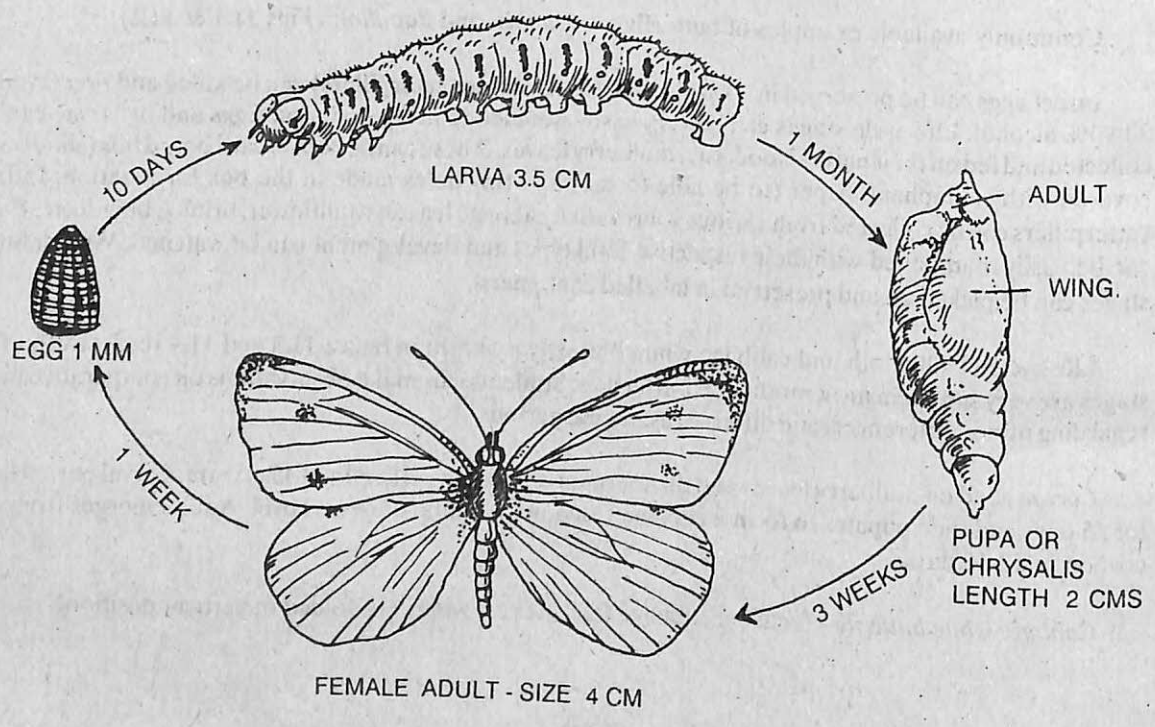


Fig. 11.4. Life cycle of cabbage white butterfly.

QUESTIONS

Q.1. What is a larva ?

Ans. A larva is free living, developing young of an organism. It does not resemble the adult and undergoes a process of metamorphosis to look like the adult.

Q.2. Which stage in the life history of a silk moth is economically important ?

Ans. The pupal stage. The pupa is enclosed inside a coccoon. The coccoon is made up of long strands of silky threads that are extracted, for making silk.

Q.3. Name an insect where larval stage is absent in the life history.

Ans. Cockroach. It has incomplete metamorphosis.

Q.4. How is the coccoon stage useful to the insect ?

Ans. It protects the developing young against unfavourable environmental conditions. Coccoons get stuck to other animals human, articles, leaves etc and are dispersed.

Q.5. Of Moths and Butterflies which are nocturnal ?

Ans. Moths.

Q.6. Beside the above point, how else will you distinguish the two ?

Ans. At rest, the wings of butterflies are folded in vertical position. In moths this is not so. Wings are horizontally spread.

Q.7. Give some characteristic features of a caterpillar.

Ans. It is a segmented, worm like, soft bodied creature. It is a voracious eater, chewing leaf or stem parts at incredible speed. For this, they have strong biting and chewing mouth parts. They have respiratory openings and walking 'legs'. They grow at a fast rate and then stop growing to pupate and undergo metamorphosis.

Q.8. A large number of insects are agricultural pests. Which stage of the life history causes damage ?

Ans. In many cases, it is the larvae that destroy plant parts by chewing or boring into the soft parts.

CORE EXPERIMENT 12

Aim : *To study fermentation by yeast and testing of the products (alcohol and CO_2) and to observe effect of temperature.*

Materials Required : Conical flask (250 ml.), one 100 ml. beaker or conical flask, delivery tube, 1-holed stopper to fit 250 ml. flask and the hole to fit delivery tube, wide test tube, sugar, glucose, lime water, potassium iodide, baker's yeast, bean or gram seeds about 20 in numbers, plaster of paris or vaseline, spirit lamp.

(a) *To observe evolution of CO_2.*

Procedure : To set this apparatus yeast culture should be prepared 2 days in advance in summer and about 7 days in advance in winter. To make the culture prepare 10% aqueous solution of canesugar (sucrose 50 grams in 500 ml. water). You may use molasses, dilute grape juice. To this add one tea-spoon full of bakery yeast, which can be obtained from a grocer's shop in dry form. Place 20 bean or gram seeds in this mixture. Cover the mixture lightly with cotton and leave the same in a warm and dark place. Set up the apparatus as shown in the figure. 12.1. Pour the culture in the conical flask up to the level of delivery tube. Keep the stopper tight. You may cover with plaster of paris or vaseline to ensure air-tight apparatus. Dip the other end of the delivery tube into a beaker containing lime water. You will observe that the lime water turns milky very fast. This is due to evolution of CO_2 during anaerobic respiration/fermentation.

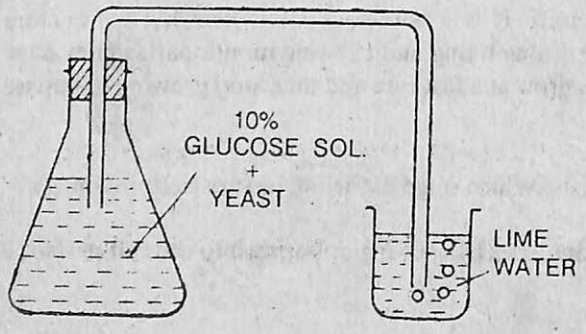


Fig. 12.1. Anaerobic Respiration

(b) *To test the presence of alcohol*

In a test tube take a small amount of supernatant liquid from the culture, after allowing the yeast cells to settle. Add few drops of potassium iodide (aqueous) and heat for a while, taking precaution not to keep the mouth of the test tube near the flame. Brown coloured crystals of iodoform will settle at the bottom on cooling. You can smell the culture for the presence of alcohol. To confirm, you can perform the same experiment with spirit diluted with half the amount of water, brown crystals of iodoform will be formed.

Precautions: Do not bring culture solution in direct contact with the flame of the spirit lamp.

(c) *To study effect of temperature*

1.) Prepare three sets of above experiment.

2.) Maintain the above experimental sets in (a) low temperature, i.e.,near zero inside a fridge or in crushed ice, (b) optimum temperature i.e., 37° C in a water bath, (c) high temperature, i.e., around 100° C in a water bath

Record observations for a, b and c with respect to lime water turning milky, not turning milky or partially milky and time taken in each case.

QUESTIONS

Q.1. Why is it essential to set the apparatus in advance ?

Ans. In order to activate yeast cells, there is need of incubation period. Once the yeast culture has established, the process of fermentation begins.

Q.2. Why is it recommended to keep the apparatus in a warm and dark place ?

Ans. To accelerate the process of fermentation.

Q.3. Why is it advised not to fill up the flask up to brim with sugar solution ?

Ans. Once the yeast is activated, it produces alcohol and carbon dioxide. To accommodate the gas produced, the flask is not filled with sugar solution. Slowly the gas moves through the glass tube into lime water.

Q.4. Why is it recommended to take supernatant fluid for alcohol test ?

Ans. The specific gravity of alcohol is less then water. Hence, the alcohol floats above watery fluid.

Q.5. Why is it not advisable to keep the spirit lamp close to the apparatus ?

Ans. The alcohol produced in the flask can catch fire.

Q.6. What is baker's yeast ?

Ans. It is culture of yeast in active form.

CORE EXPERIMENT 13

Aim : *Testing urine for normal and abnormal constituents (Urea, sugar, albumin and bile salts).*

Why do we test urine ?

Urine, which is a product released from the kidneys, is an important indicator of normalcy of body metabolism. The nephrons in the kidney are in close and rich contract with the blood. The blood brings metabolic wastes from all over the body and gets filtered in the kidney, taking back with it the important constituents and leaving behind the nitrogenous wastes. Thus, a correct analysis of urine for its normal and abnormal constituents is an important aspect of medical examinations. A large number of diseases are diagnosed with the help of results based on urine analysis.

Urea, uric acid, ammonia and creatine are normal constituents of urine. When glucose, acetone bodies, bile, blood or albumen appear in the urine, medical consultation becomes essential. These constituents can be added to water (distilled) by the teacher and students can perform tests to detect them by treating it as a urine sample.

Material Required : Test tubes, test tube rack, urine samples or water containing the components to be detected, test tube holder, burner, various chemicals as mentioned for the different tests.

Procedure :

(A) *For normal constituents of urine*

1. Urea : Add 0.2 gm of urea to 5 ml of water in a test tube.
   (a) Effervescence test : 3 ml of sample + few drops of alkaline sodium hypobromide solution. Marked effervescence confirms urea.
   (b) Urease test :5 ml of sample + 4/5 drops of phelphthalein - Pink colour

To above, add dropwise 1% acetic acid solution, till pink colour disappears.

Now add 3 ml of 2% urease solution or just a pinch of urease powder. Allow it to stand at 37° C or room temperature for about 20-30 mts. Reappearance of pink colour confirm urea. Urease breaks down urea to ammonium corbonate which increases the alkalinity, causing the reappearance of pink colour.

2. Ammonia : Add a few drops of phenophthalein to 3 ml. of the sample. The solution turns milky. Add a pinch of urease, solution turns red or pink. Urease breaks urea to ammonia, which causes the pink or red colour.

3. Uric acid : For this test, the white part of dried bird excreta may be used.

Muredixe test : Take small amount of above sample in an evaporating dish + few drops of conc HNO3 (Nitric acid). Evaporate over a slow flame till dry. A red or yellow residue is left. Cool, add a drop of 2% ammonium hydroxide solution. A reddish purple colour indicates uric acid.

Uric acid is oxidised to dialuric acid and alloxan which condenses into alloxanther. Alloxanther reacts with amm. hydroxide to form the purple compound murexide.

4. Creatine - Jaffe's test.

4. 5 ml of urine sample + 1 ml of saturated solution of Piccric acid + 1 ml of 10% NaOH (Sodium

hydroxide) The solution turns red, indicating presence of creatine. The red colour is of creatine picrate.

(B) *For abnormal coustituents of urine .*

Glucose appears in the urine of diabetic patients.

Bennedicts test : 0.5 ml (or 8/9 drops) of urine sample + 5 ml of Bennedicts reagent.

Boil for 3-5 min. A green, yellow, orange or red ppt appears depending on the amount of glucose present in the urine. Orange, red colour indicate higher contents of glucose. Simple sugars such as glucose have the property of reducing copper sulphate in alkaline solution (as in Bennedicts) into insoluble ppt of Cuprous oxide which may be yellow, orange or red in colour.

2. Albumen : When present, the condition is termed Albuminuria.

Heller's test : To 1 ml of conc. nitric acid add urine sample by means of a dropper from the side of the test tube, so that it forms a layer on top of the nitric acid. A whitish ring formed at the junction of acid and urine indicates presence of albumin.

3. Bile salts

Hay's test : Take two beakers, one with the urine sample, the other with plain water or urine sample without bile salts. [Bile can be collected from gall bladder of frog, bird or fish]. Sprinkle sulphur flowers on the fluid surface in both the beakers. The sulphur flowers will sink to the bottom if bile salts are present, due to lowering of surface tension. They will float otherwise.

NB - Sulphur flower or flower of sulphur is a fine powder obtained by melting solid sulphur and then allowing it to vapourize.

4. Acetone bodies : These appears in the urine when fat metabolism is disordered and are often formed in conditions of diabetes mellitus and in cases of severe starvation. The patient is said to have ketonuria as Ketone bodies are present in the urine.

Rothera's test :- Add excess of solid ammonium sulphate to 5 ml. of urine sample so as to saturate it. Add one crystal of sodium nitro prusside. Carefully run down the sides of the test tube, liquor ammonia, so as to form a layer on top of the urine. A permanganate coloured ring form at the junction of the urine and ammonia layer. The colour spreads uniformly on shaking the tube.

5. Blood : Blood may appear in the urine as R.B.C. and its presence is termed 'Hematuria'.

Benzidine test : Prepare reagents (a) + (b)

(a) Saturated solution of benzidine in glacial acetic acid.

(b) Hydrogen proxide.

Mix equal parts of (a) and (b) in a test tube and add this mixed reagent to an equal volume of urine. A green/blue colour confirms presence of blood. The solution when allowed to stand for five minutes, turns purple or brown.

QUESTIONS

Q.1. Why is urine test important ?

Ans. Urine consists of metabolic wastes. These are excreted in specific quantities under normal physiological conditions. These constituents impart specific physical and chemical characteristics

to urine. Any major deviation of these characteristics indicate towards metabolic disorders which can then be diagnosed and treated.

Q.2. Where is urea formed ?

Ans. In the liver.

Q.3. Which are the constituents which when excreted in excess indicate abnormality ?

Ans. Glucose, albumen, bile salts, acetone bodies and blood.

Q.4. Why is it that glucose is usually not found in normal urine ?

Ans. Glucose is an easily available, easy to utilise metabolite that releases energy. Being a molecule much in demand, it is completely reabsorbed in the nephric tubules, to be returned to blood.

Q.5. Urine is more watery in rainy or winter season and not so in summer. What is this due to ?

Ans. This is a homeostatic mechanism to maintain right concentration of body fluids. During rains, the the body does not need to conserve excess water as outside conditions are characterised by excess humidity. In summer, environment is dry, there is more loss of water from our body as evaporation. There is need to conserve water, thus there is more reabsorption of H_2O by the tubules, making the urine less dilute as compared to that during rain or winter.

Q.6. In spite of somewhat constant features of urine, do you expect small variations in its constituents during the 24 hours ?

Ans. Yes, because it depends upon what all one has eaten and the kind of metabolism the "eaten" items undergo. It also depends upon various environmental factors, mental and physical state of a person.

Q.7. As far as normal constituents of urine are concerned, will you find lot of variation on analysing urine from adult males, females and children or infants ?

Ans. No, because basic metabolism that go to the formation of urine are same for all.

Q.8. Which animals excrete solid form of nitrogenous waste ? Why ?

Ans. Birds and reptiles secrete uric acid as solid crystals. Their mode of life is such that they need to conserve all the water they take in, for e.g. desert animals (scorpions) also excrete solid waste.

Q.9. What is the source of nitrogenous waste in our bodies ?

Ans. These are breakdown products of protein metabolism. The excess of amino compounds from amino acids get converted to urea or uric acid.

Q.10. Which other secretion of our body also contains nitrogenous wastes such as urea or uric acid ?

Ans. Sweat contains these.

CORE EXPERIMENT 14

Aim : *To study the effect of antibiotics on micro-organisms.*

Material Required :- Nutrient medium, hay straws, pipette, petridishes, Penicillin tablets or vials, Newspaper sheets or brown paper sheets, sprit and sprit lamp, flask, distilled water, pressure cooker, heating arrangement - kerosene oil stove or gas stove or electric stove, Hot air oven.

Procedure :

(A) *Preparation of culture medium for bacteria* : You can purchase sterilized culture medium from the market in sterilized culture tubes or petri-dishes. You can also prepare the nutrient medium in the laboratory by using following ingredients

Meat extract = 3 gm
Peptone = 10 gm
NaCl = 5 gm
Water = 1litre

Agar-agar = 18 gm to 22 gm depending upon season.

Dissolve meat extract, peptone and NaCl in half litre of water and heat for complete homogenous solution. In another one litre flask transfer agar-agar and rest of the half litre of water. Allow to heat gently and shake the flask till the agar-agar dissolves and forms a clear solution. Transfer the meat extract etc. into this flask and autoclave or pressure cork for 30 min at the pressure of 15 lbs per square inch.

You can change the quantity of agar-agar when desired. In winter use less quantity and in summer use more since agar agar helps in setting of the medium, the variation in quantity is necessary.

(B) *Preparation of bacterial culture* : For the laboratory purpose, you should select bacterial culture which is harmless. For this you may use yagurt (curd) as a source of *Bacillus lactis*. You can boil few pieces of hay straws in a beaker which contains about 200 ml of water. Cool and keep aside the beaker for 2-3 days. *Bacillus subtiles* will grow in this water. A drop of this culture is enough for the experiment.

(C) *Sterilization of glass ware :* Wash and dry about a dozen of Petridishes pairs (4 cms diameter) and place them in the hot air oven at 120° C for two hours. Cool and use next day.

Transfer of sterilized nutrient medium into sterilized petridishes : In a clean dry place in your laboratory spread a brown paper or newspaper sheet and sprinkle spirit to make the paper free from infection. Pour a small quantity of sterilized nutrient medium from the flask which is not yet cool enough for the medium to settle in the flask. Cover the bottom of the petridishes with the medium. Wrap these petridishes with sterilized paper and place till the medium has set into semi-solid form.

(E) *Inoculation of Bacterial Cultural into the nutrient medium :* With the help of a pipette pour out few drops of bacterial culture and sprinkle over the nutrient medium in the petridishes. You can use your finger tips for this purpose or even inoculation needle can be used. Transfer these petridishes back in the paper wrapper and place at a dry and moderate temp. say 37° C. You can use incubator, oven or switch on a bulb

to maintain the temperature. Leave as such for 2-3 days.

(F) *The effect of Penicillin on Bacterial growth :* Dissolve penicillin powder in distilled water (2 ml). Cut filter paper into 1/2 cm pieces. Soak these pieces into penicillin.

Place the penicillin soaked filter paper discs four each in a petridish and place them into incubator or in a dry dark place in the laboratory. Keep two petridishes without Penicillin, these act as control. Wait for 2-3 days.

(G) Perform similar experiment with bread mould (Rhizopus)

Observations :

After 4-5 days you can open the wrappers one by one and look into the petridishes. You may observe proper growth of bacterial culture in petridishes but in the petridishes treated with penicillin show a clear zone of subduced growth or no growth. This is zone of inhibition preferably seen around the penicillin soaked discs.

Results : Antibiotics check the growth of micro-organisms, like bacteria and fungi.

Disscussion : Antibiotics are the secretion produced by some micro-organisms. These in turn inhibit the growth of many other micro-organisms. When bacterial culture is grown in a nutrient medium, the culture develops, but on mixing antibiotics the growth of the bacterial culture is suppressed. This is confirmed in the control petridishes.

Precautions :

1. While selecting bacterial culture, always use harmless bacteria in the laboratory.
2. To avoid contamination, sterilization of all the items is essential.
3. Label all the apparatus properly.
4. Select a clean, dry place for sterilization, inoculation and incubation.

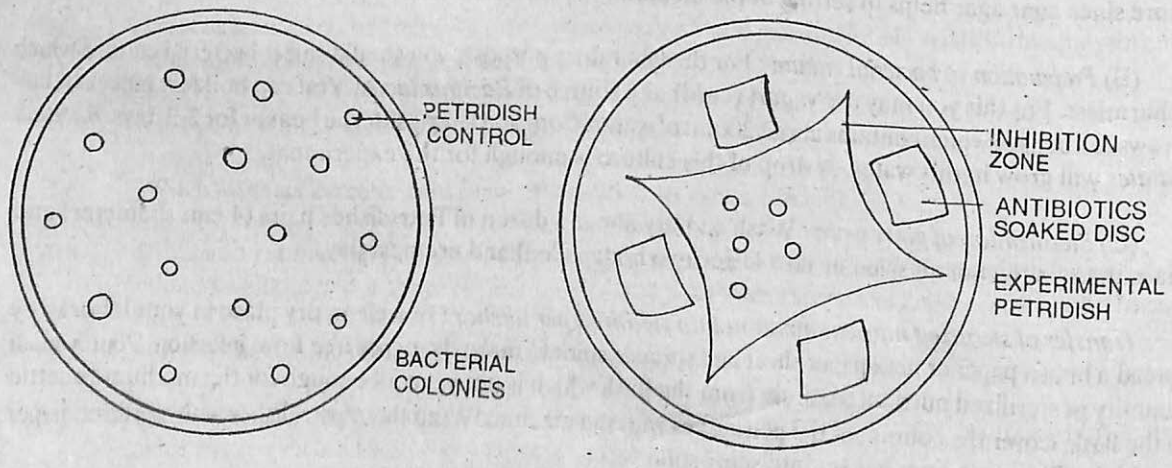


Fig. 14.1. Effect of antibiotics on the growth of micro-organisms

QUESTIONS

Q.1. What are antibiotics ?

Ans. Antibiotics are secretion of microrganisms which inhibit the growth and activity of other microrganisms.

Q.2. Why is sterilization of apparatus essential ?

Ans. To prevent contamination of a variety of microbes sterilization is essential. Thus only the desired mirco-organisms can be cultured in this medium.

Q.3. Name two antibiotics and their application.

Ans. Penicillin - for respiratory infection, tuberculosis
Streptomycin - for viral infection, boils.

Q.4. Why is Penicillin not effective on all types of microrganisms.

Ans Penicillin is not effective on all types of micro-organisms because growth of all microrganisms cannot be suppressed by one type of antibiotic, e.g., Salmonella typhae can be checked by Chloromycetin.

Q.5. How are antibiotics available commercially ?

Ans. In the form of tablets, capsules, vials.

Q.6. Name some antibiotics that are used for other purpose besides medication.

Ans. Griseofulin is used to control bean rust.

Q.7. Why is it not recommended for a prolonged use of antibiotics.

Ans. Long term use of antibiotics may lead to development of resistance of pathogenic microrganisms.

INVESTIGATORY PROJECTS

Investigatory Project 1

To study Mendelian Traits in garden pea.

Material - Seeds of Pea about 100 gms. Garden soil with appropriate amount of manure. Patch of garden bed or 6 earthenware pots of 25 cm. diameter.

Method - Select healthy seeds from the sample. None down few of contrasting characters and make a record, e.g. texture of the seed, size of the seed and colour, soak these seeds in water for about 3-4 hours to check if all the seeds are healthy. Seeds with any infection will not retain the same shape. Thus they can be removed. Depending upon the available space, sow these seeds in the piece of bed or in pots (not more than 5 seeds per pot). Keep these pots in well aerated and sunny place. Pea is grown in the month of October and November. The fertilizer is not required in large quantity, because of legume. Water is required in optimum amount. No flooding is recommended. You should avoid the infestation of the plant from insects, beetles, etc. Wait till the flowers are produced. Since the plant is a leaf tendril, a suitable support is necessary. May be you can use bamboo sticks and a small piece of thread to tie the young plants. All these pots are numbered by metallic lables.

Once the flowers are produced, you should note down the position colour of the flowers. Allow the self pollination by avoiding insects. For this you may have to cover the plants with a fine muslin cloth or wire mesh can be substituted. Can you give reason to this ?

Wait till the pods are developed. Look into the texture of the pods, colour and size as well.

Once the seeds are ready, you can collect seeds per plant. Once again you can check the colour, size and texture of the seeds. In order to complete the experiment, you will have to wait till late February.

Observations - The seeds for germination produce small seedlings. These seedlings can develop into full size plants in due course of time. During this period, a special care is required. The seeds that were selected had different phenotypic characters. The aerial portion also shows phenotypic variation, as is evident by the size of the plant, leaves borne. Flowers are produced on axillary and terminal positions. The size of the pods varies. In some pods the surface is smooth and in some the hairy growth is persistent. These observations are recorded in a table for each seed/plant.

Contrasting Traits

	Dominant	*Recessive*
Seed shape	No. of Seeds	No. of Seeds
Seed colour	Round	Wrinkled
Size of the plant	Yellow	Green
	Tall	Dwarf
Colour of the flower	Red	White
Position of the flower bud	Terminal	axillary

Size of the pod Surface of the Pod	Long Smooth	Small Hairy
Total no. of seeds harvested per plant Seeds shape Seed colour Seed size		

Results - The seeds when sown and harvested after the crop is fully grown, there is some variation in respect to seed colour, size and shape.

Discussion - The garden pea was selected by George Mendel for his experiments on heridity. In order to verify these characters the seeds are grown every time. The results obtained in the crop are showing variation. This is mainly because, some dominant characters in the seeds were heterozygous. When selfing was allowed, the recessive characters have emerged such as when seeds with smooth texture are sown, some seeds are produced with wrinkled surface. Similarly you can give reasons for the other traits also. In order to prove Mendelian principles on inheritance your experiments can be continued for two or three years. The recessive characters are always homozygous, whereas, the dominant phenotypic characters can be homozygous or heterozygous.

QUESTIONS

Q.1. Why did Mendel select only few characters for his experiments ?

Ans. He could select only those which showed contrasting features in a given trait. Secondly, studying few characters at a time was less confusing and proper conclusions could be drawn.

Q.2. What is the cause of reappearance of recessive characters in the seeds that originally exhibited dominant features ?

Ans. The seeds were not homozygous for the dominant feature. They were heterozygous. Since segregation of genes is at random in the gametes, in some zygotes the two recessive genes came together at fertilization. Thus the recessive features reappeared in a homozygous condition.

Investigatory Project 2

Emasculation of flowers.

(A) *To study the effect of emasculation of flowers and to compare the results with that of selfed flowers.*

Material - Garden pea seeds, earthenware potsh with garden soil, forceps, polythene bags, tags.

Method - Growth of plants :

The plants are raised from the seeds with recessive and dominant characters separately. One or two seeds are grown in each pot. The pots are labelled for record purpose. The pots are placed in an area, where, not many students are working. This avoids confusion and mishandling by the other students.

Emasculation of flowers - The pea flowers can develop by self pollination and cross pollination. In certain experiments cross pollination is essential. Self pollination is considered in the experiment where contrasting characters are controlled . In order to encourage cross pollination in such flowers, removal of stamens and pollen grains is required. This should be done before they are ready for dissemination. Generally the self pollinated flowers have corolla that covers anther lobes and stigma. The anther lobes are removed from these flowers by the help of forceps at an early stage. The cross pollination can be carried out by disseminating pollen grains from one flower to another with the help of forceps. These flowers are then covered by polythene bags and tags are tied. The pods are developed after few days. The observations are recorded in a table.

Observations - The seeds collected in these experiments resemble with the original type or show some new characters.

Results - The development of new characters indicate that some of the seeds were hybrid variety and some were homozygous, dominant and recessive.

Discussion -The dominant characters seen phenotypically are either of pure dominant alleles or of heterozygous combination. Only the recessive characters can be seen phenotypically when they are in homozygous combination.

In case of emasculation technique the cross pollination can be carried out between two contrasting characters and the result can give the actual combination while selfing the desired characters are allowed for selfing to prove law of segregation. The reappearance of recessive characters can be seen in these experiments, where there is probability of two recessive genes to come in the progeny. You can collect data for several other characters and use other crops when selfing is possible like groundnut.

QUESTIONS

Q.1. Why is emasculation technique useful ?

Ans. Emasculatisn is helpful as it prevents self pollination and cross pollination becomes inevitable.

Q.2. What are the advantages and disadvantages of selfing ?

Ans. Advantage : In self pollination, pure breeds with respect to a particular trait can be obtained. If this trait is a useful one, it can be maintained for many generation by selfing.
A disadvantage is that while some good trait can be maintained, there is lack of variations in general. Absence of variations for many generation in a given plant may prove harmful for the plant in due course of time. Variations are useful as they allow scopes for adaptations.

Investigatory Project 3

Population survey to identify human phenotypic characters-rolling of tongue, fused ear lobes and colour blindness.

Inheritane of characters in human beings obeys the same laws as in other animals. Broadly speaking the inheritance may be of two types the autosomal and the sex linked. Colour blindness is an X-chromosome linked trait while tongue rolling capacity and presence or absence of fused ear lobes are examples of autosomal inheritance. In these investigations, concerning population genetics, it is desirable that large samples be considered for arriving at satisfactory results.

(A) Tongue rolling and fused ear lobes.

One can find out how common or uncommon these traits are. The sampling must be done as randomly as possible,in order to be truly representative of a population. For this, care should be taken not to include members of the same family in one sample. Observe the traits as given in the figures below and tabulate as shown. Fig. 3.1, a & b.

1. No. of tongue rollers =
2. NO. of non rollers = } Sample size at least 50.
3. Percentage of rollers =

Similarly tabulate for attached and free ear lobes. After finding the frequency of occurrence of these traits, one can collect further information by a family-wise data. Families with 3-4 children should be considered. One can find out whether these features are dominant or recessive.

Clue - For examples if tongue rolling is dominant, a marriage of two tongue rollers may produce children who cannot roll the tongue. If it is recessive, a marriage of two tongue rollers produces only tongue roller children.

Colour blindness - This trait is inherited through a recessive gene on the X-chromosome and is much more prevalent in men since they have only one X-chromosome. In women, even if they carry the gene, the effect is marked by its normal counterpart on the other X-chromosome. A woman suffers the abnormality in a homozygous recessive condition.

Colour blindness often forms a part of a routine medical examination for school children and industrial workers. It is very important in occupations like train guards, railroad workers, drivers, traffic policemen, etc. This is because signals and traffic lights are red and green. A common defect is the inability to differentiate between these two colours. Abnormality in the rods and cones of the retina is responsible for such defects in vision. Colour blindness may be of the following types :

a) Red blindness or protanopia.
b) Green blindness or deuteranopia.
c) Blue blindness or tritanopia.

Simple test for colour blindness can be performed by using colour plates called 'Ishihara plates'. These plates have figures outlined in dots of primary colours (red, green, blue) printed on a background of dots in

confusion colours. The normal person can see all the figures at a glance, but the colur blind person makes mistakes.

From the errors he makes, the type of defect may be detected.

The cards may be prepared and shown to both boys and girls. The test should be conducted in broad day light. The cards or charts should be held at a distance of 40 inches or one metre. The card should be studied for about 15 seconds and not more.

The parents of the colour blind child should also be tested. From the data, The pattern of inheritance can then be worked out.

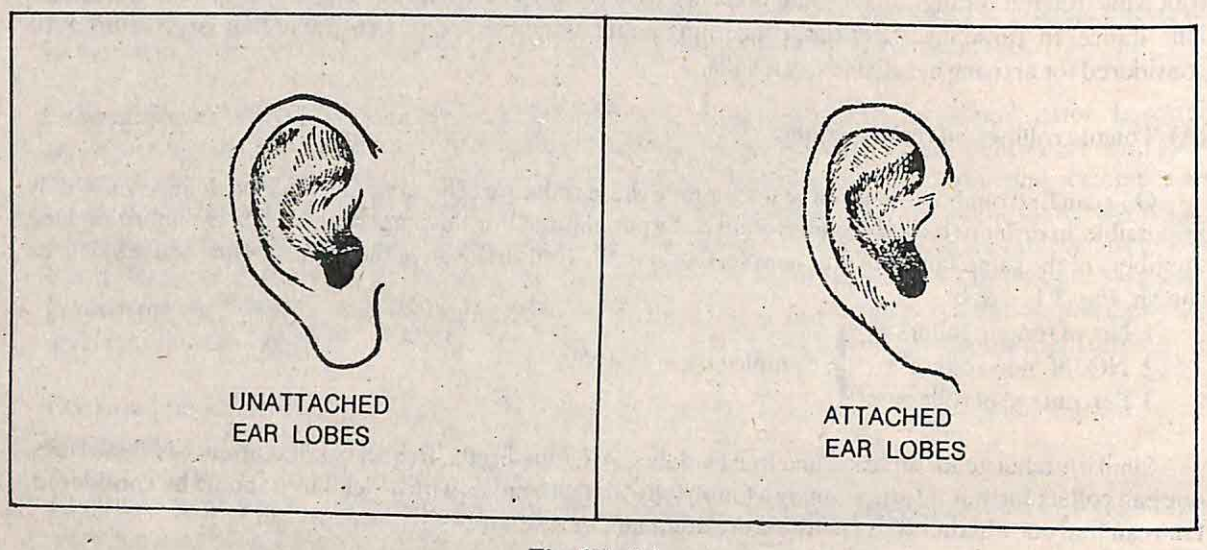


Fig. 3.1. (a)

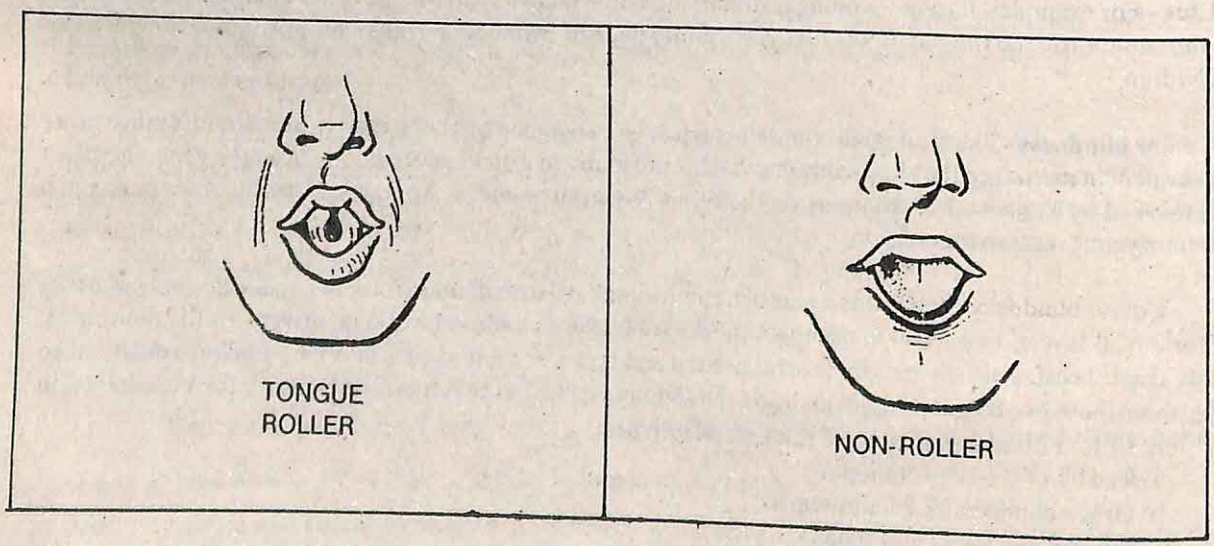


Fig. 3.2. (b)

QUESTIONS

Q.1. What is the significance of investigating with human phenotypic traits ?

Ans. a) We ourselves being human, we should know how certain traits are inherited.
b) One can compare patterns of inheritance among families, with other animals.
c) The investigation helps in learning about the methods of studying population genetics.

Q.2. Of three traits studied, which hinder in normal life and which do not ?

Ans. Colour blindness is a hinderance to some extent. It is more important for drivers who cannot get a liscence if they have this defect. Tongue rolling and fused ear lobes do not hamper with any activity.

Q.3. It both parents can roll their tongues, will all their children also have this capacity ?

Ans. No, because if tongue rolling is dominant, the parents may be heterozygous rollers, and may have children who are not rollers. (Mendelian segregation)

Q.4. Which cells are defective in the eye, that result in colour blindness ?

Ans. Rods and cone cells.

Q.5. Why is it important to consider a large sample for such studies ?

Ans. Conclusive results are possible only when data or observations are statistically dependable. This is possible only with large number of observations.

Investigatory Project 4

Aim : *Study of frog / toad ovary during breeding season to identify the stages of maturation of ova.*

Requirements - Female frogs of varying ages, dissection instruments, microscope, slide, coverslips and amphibian ringers solution or 0.6% saline.

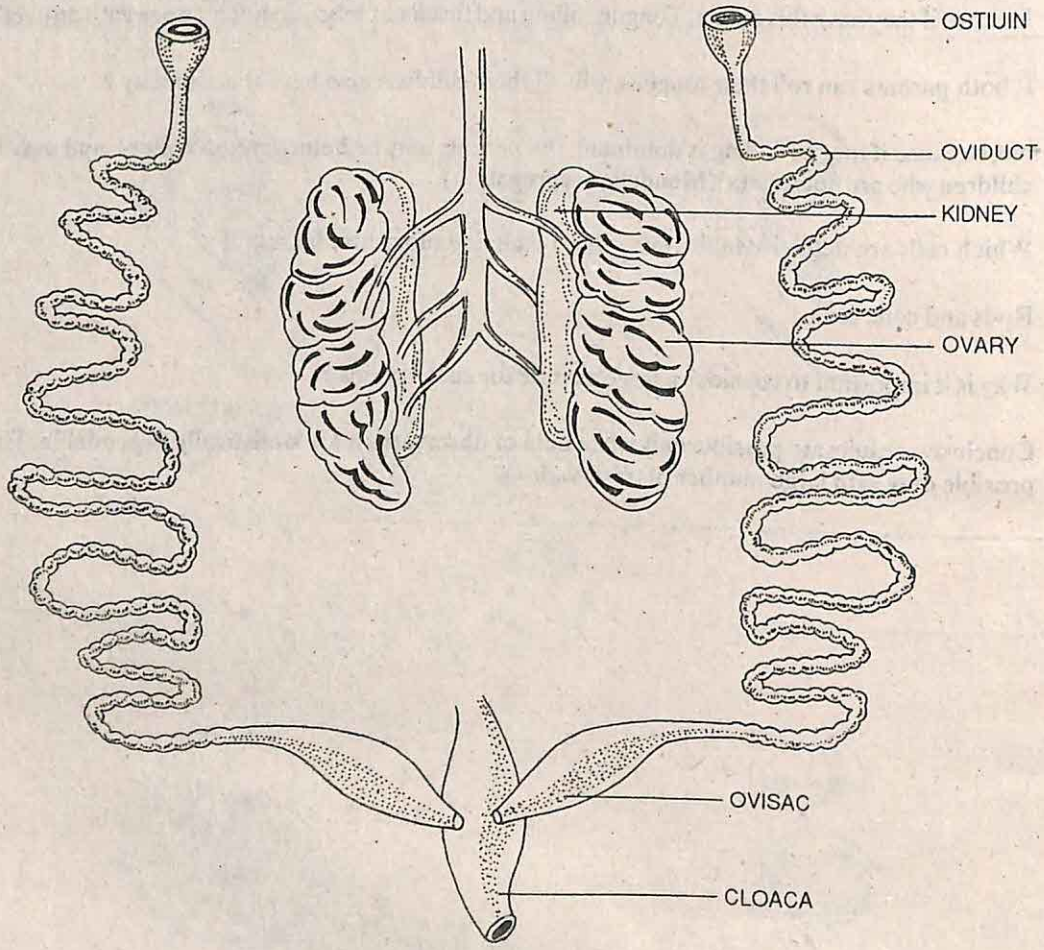


Fig. 4.1. Female reproductive system

Observations - Observe the diagram for female reproductive system. You will notice that ovaries are lobulated, sac like structures attached ventrally to kidneys. In the breeding season ovaries become enlarged with large number of follicles, each follicle with countless dark coloured ova. The oviducts are coiled tubular structures on either side of the ovaries. They begin anteriorly near. The under surface of lungs, with an opening- the ostium. The oviducts continue down and open separately into the cloaca. Just before this opening, oviducts swell into structures called ovisacs where mature eggs are stored temporarily. The oviduct secerte an

albuminous substance with which the eggs are coated as they pass down along the oviduct. The albuminous substance has the property of absorbing water and swell enormously to form a coherent mass that form the spawn. Eggs are released in the body cavity by rupturing of the thin covering of the ovary. They are carried forword to the opening of the oviduct assisted by the pressure of the fore arms of the clasping male. Finally eggs are released in large clusters into the cloaca and then into the water when fertilization occurs.

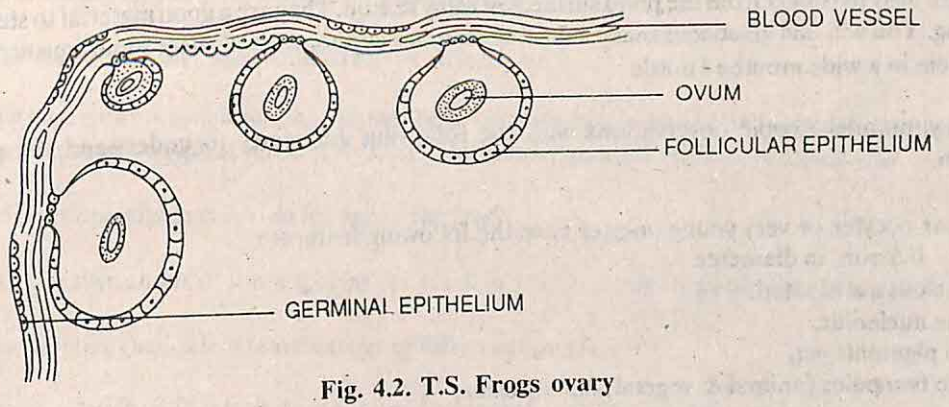


Fig. 4.2. T.S. Frogs ovary

The above diagram gives you an idea of the histology of frogs ovary. In a transverse section, the ovary shows a single layer of germinal cells, some of which differentiate into mature ova by the process of oogenesis. One of the many cells of the follicles is destined to form the future ovum and the remaining cells are gradually modified to form a thin investment the follicular epithilium around it. The folliar cells help in the growth of the oocyte by actively secreting substances that are absorbed by the oocyte. Within the follicle a vitelline membrane is secreted by each ovum around itself.

The young oocytes start growing after tadpoles metamorphose into young froglets. One or two year old frogs do not have mature eggs, but in the third year maturation starts and there is rapid growth. A young oocyte is about 50 microns in diameter while a fully grown oocyte is 1000 - 2000 microns in diameter.

Procedure :- Procure female frogs - preferably of different ages. You can collect large frogs in rainy season or just prior to rainy season. These will have mature eggs. Collect smaller frogs in winter or those that have just completed metamorphosis. Female frogs can be identified by the absence of copulatory pads and vocal sacs, present in the males.

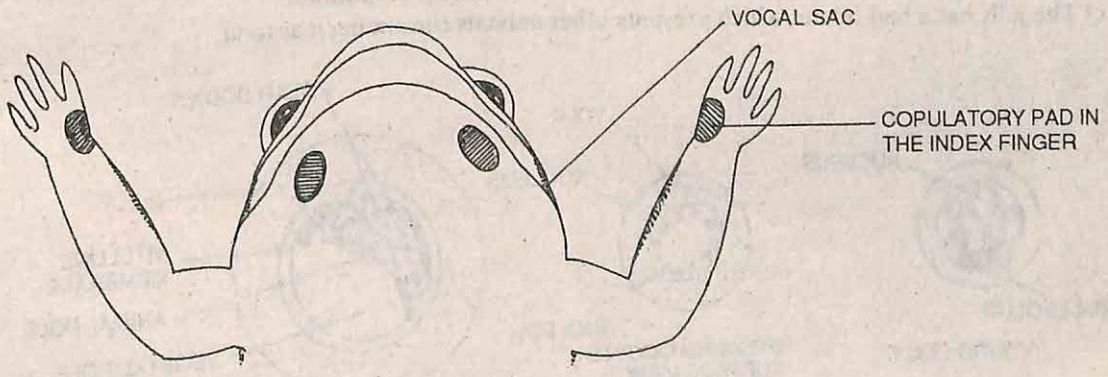


Fig. 4.3. Male Frog

Use chloroform and kill the frogs. Expose ovary. Pour 0.6% saline to keep the tissue alive. Rupture the ovarian membrane, pinch off a small portion of the follicles. Place this material on a clean slide. Pore a few drops of the saline solution, place a coverslip. Observe under low as well as high power. You can observe ovarian tissue from a fully mature frog and also from others that are not yet matured. A comparison of fully mature ova with young ova will give you a better idea of the changes that occur during maturation. Frog spawans are easy to collect from the pond surfaces in rainy season. They are a good material to study mature eggs of frog. You will find gelatinous masses floating on water surface specially near some aquatic vegetation. Collect them in a wide mouthed bottle.

Tally your microscopic observations with the following guidelines to understand the process of maturation.

1st year oocytes or very young oocytes have the following features :

a) 0.1 - 0.5 mm. in diameter.
b) Nucleus not bloated.
c) One nucleolus.
d) No pigments yet.
e) The two poles (animal & vegetal) not yet clear.

3rd year or mature eggs have the following features

a) 1.0 - 1.5 mm in diameter
b) Nucleus bloated, because of increase in nuclear sap. The structure is also called a germinal vesicle.
c) Nucleolus enlarged & conspicous, often many nucleoli are formed that become localised on the periphery of the nucleus.
d) A growing ovum is surround by follicle cells
e) The upper part (animal pole) start appearing darker because of pigments. The animal pole have the cytoplasm and the nucleus. Pigments start appearing when oocytes are about half their final size, the pigment colour varies from brown, dark brown to black, depending upon their amounts.
f) The vegetal pole appear lighter in colour and is a larger part of the total surface. There are hardly any pigments & this half contains the yolk. A mature amphibian egg contain about 45% protein, 25% lipids, 8.1% glycogen in the yolk and the rest ie about 20% is the cytoplasm.

Observe the orientation of eggs in the gelatinous mass. The jelly has several functions, such as

a) It glues together the eggs and fertilization of a large number of eggs occur simultanously.
b) It keep eggs floating, thus sufficient oxygen is avialable for respiration.
c) The jelly has a bad odour, which prevents other animals consuming it as food.

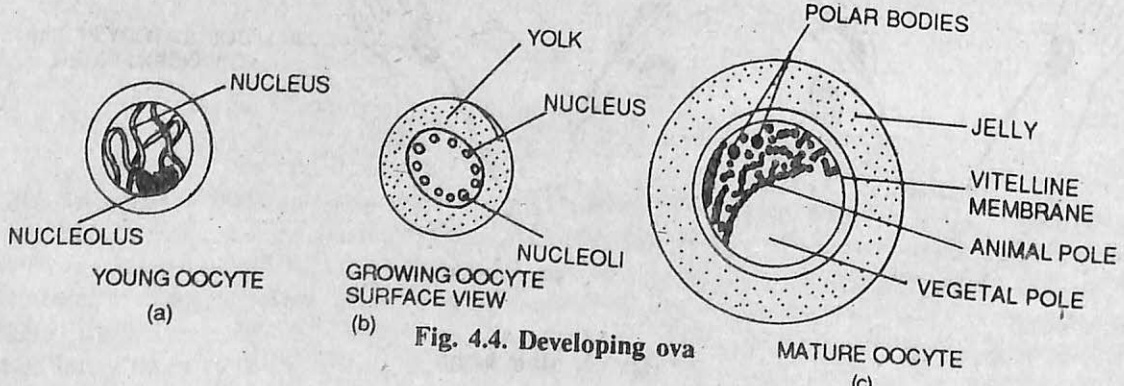


Fig. 4.4. Developing ova

QUESTIONS

Q.1. Why is the frog's egg described as a polarized structure ?

Ans. Frog egg has unequal portions. The upper part is the cytoplasmic part & is dark due to pigments. The lower half is rich in yolk & has very little pigment.

Q.2. Why do we pick an example like frog to investigate ?

Ans. Frogs are easily available and in large numbers. They are easy to handle and they are harmless animal. They represent a typical vertibrate. They exhist both aquatic & teristrial adaptations.

Q.3. Is size a dependable criterion for age of the frog ?

Ans. No., because adults of some species are small in size & young frogs of other species are large.

Q.4. How will you then select female frogs of different age groups ?

Ans. This is actually difficult & there is no fool proof method, unless one keeps a watch on the metamorphosing tadpoles. But in general a given pond or habital is inhabited by the same species & therefore small & large frogs can be taken as criteria for young & adult animals.

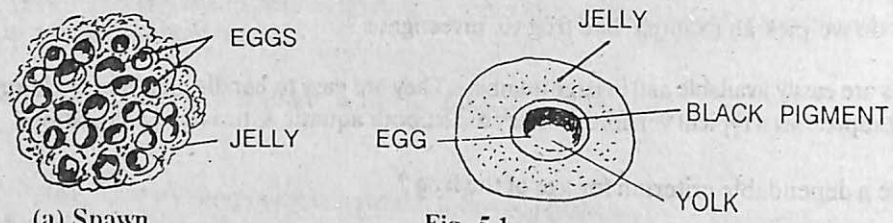
Q.5. Fertilization is external, then how does mating help ?

Ans. By the act of mating, the female is helped to release the cluster of eggs. Also as the sperms are released in the colse vicinity of the eggs, a large number of fertilization are assured.

Q.6. Why do we use 0.6% saline or amphibian Ringers ?

Ans. To observe any living tissue outside the body of the animal, it needs to be maintained in a fluid which is similar to that of the body fluid, i.e. an isotonic medium. 0.6% saline or Ringers solution provide this condition. The tissue remain undamaged for considerable time.

Investigatory Project 5



(a) Spawn

Fig. 5.1

(b) Egg.

Aim : *Study and sketching of certain stages in the life cycle of frog from fresh and preserved materials.*

Material -Fresh material, sources, collection and preservation.

1. This material can be obtained from a a well maintained froggery. The water from the froggery can be collected during breeding season, for spawns. Spawns, in their natural medium can be observed for changes occurring during development, inside the lab, under dissection microscope. Major changes like hatching, appearance of limb buds, tail, etc. can be observed.

2. From the above material, various well defined stages can be picked up and preserved in small separate tubes with corks.

3. If a froggery cannot be approached, fresh material can be had in rainy season from ponds, pools and other small watery bodies which has some vegetation. Spawns are recognised as a frothy floating mass. The eggs have dark spots on them and look black or dark grey. Frog spawn need not be confused with fish eggs, as the latter are very very minute and appear as minute white spks. Frog's eggs are longer than fish eggs, individually. In case of doubt, the spawn an always be examined under a lens for confirmation. Similarly tadpoles can be collected. Tadpoles collected at random can be quickly separated according to their developmental stages and then preserved.

All these stages of frog material can be preserved in 7 to 8% formalin, which is also a fixative. In order to identify various stages, student can take help from preserved specimens. Diagrams are given below, along with approximate time.

About 2 mm. across (i.e. the egg without jelly) yellow yolk is visible in the lower third of the egg.

The embryo at day 7 is about 4 mm. in length, can be observed as an elongated structure with a head lobe and a tail. This is how an embryo appears before hatching. (Fig. 5.2).

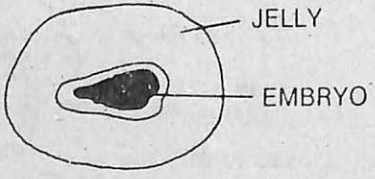


Fig. 5.2. Embryo before hatching

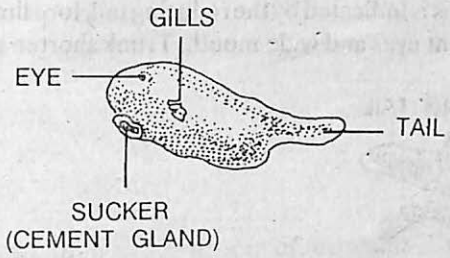


Fig. 5.3. Embryo on hatching

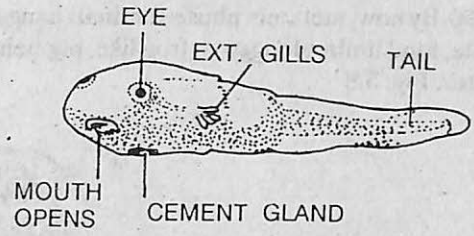
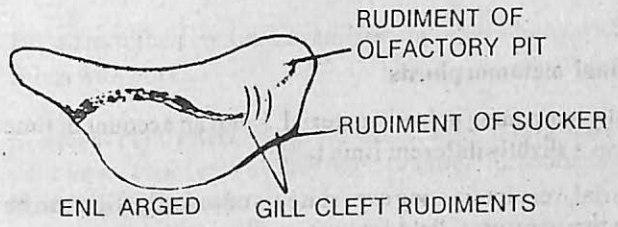
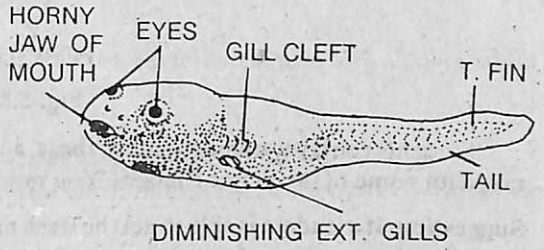


Fig. 5.4. Mouth opens



(A) gill delf stage



(B) gill cleft stage

Fig. 5.5.

Fig. 5.3 is the appearance of tadpole on hatching. (8 mm. in length) It is more elongated now. In fresh material, under low power, blood circulation in the gill capillaries can be seen. Embryo in fresh material is still attached on surface of spawns. Fig. 5.3.

The mouth opens, and cement gland (sucker) is reduced. At this stage, the fresh material is a free swimming and feeing structure Length = 7/8 mm. Fig. 5.4.

Day 22-The external gills are gradually replacedd by 4 pairs of gill clefts. Mouth has developed horny jaws. The tadpole is increased in lenght Fig. 5.5 (A) and (b).

Day 28-The front part is horny, mouth is more enlarged. A fold of skin called the operculum grows over the front part, enclosing the gill slits. In fresh material, water is taken in (for breathing) through mouth, and can be seen, coming out through a spout on the left side.

Length = 11 mm.

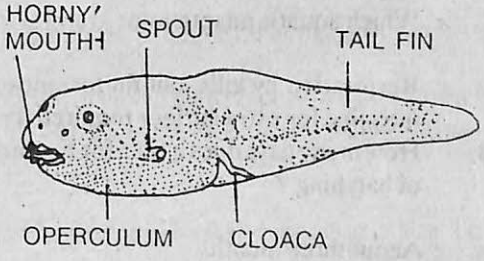


Fig. 5.6. Operculum stage

Day 50-Sufficient growth. It is no more a slender fish like body. A pair of hind limb buds are visible. Tail is more well defined, and can be seen clearly through the fin. Fig. 5.7.

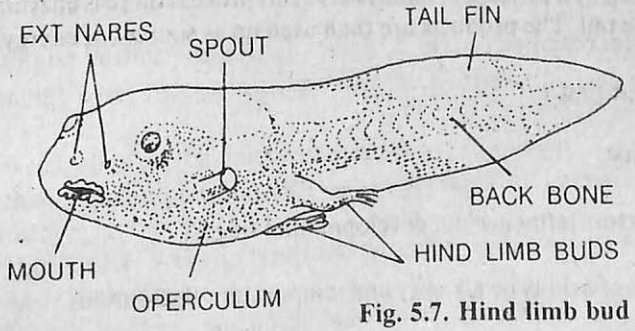


Fig. 5.7. Hind limb bud stage

Day 90-By now, metamorphosis for final changes have set in. This is indicated by the reducing tail, fore limbs visible, hind limbs elonggted, frog like, pigmentation. Prominent eyes and wide mouth. Trunk shorter and stouter. Fig. 5.8

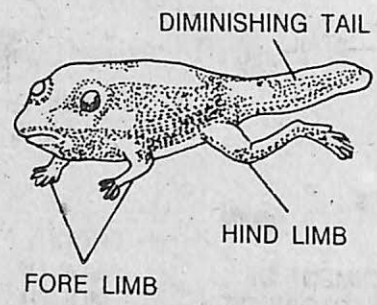


Fig. 5.8. Final metamorphosis

Students can try watching some of these, actually happening in fresh material. Keep an account of time taken for some of the major changes. You may have a slightly different time table.

Suggestion- If a student is able to get the fresh material, very interesting experiments or investigation can be done by exposing the embryonic stages to varying temperatures, light intensities, chemicals, etc.

Note-The lengths of the tadpole at various stages are those of a particular species at a given temperature. Hence these lengths are not to be considered as common features for all frogs, keeping in mind the variations that occur in different species.

QUESTIONS

Q.1. What is a tadpole ?

Ans. It is the larval stage of frogs life cycle. It is free living, an active feeder, does not resemble the adult, is adapted to aquatic life and changes to the adult like animal by a process of metamorphosis.

Q.2. Which aquatic adaptatioins are vital to the tadpole ?

Ans. Respiration by gills, tail fin for movement, horny jaws for chewing vegetative matter and long intestine for extra surface required for digestion of plant matter.

Q.3. How many days does it take for a common Indian frog to complete metamorphosis, from time true of hatching ?

Ans. About three months.

Q.4. The tail gradually diminishes. What actually happens to it ?

Ans. The tail material is used up by a process of autolysis. In this process there is enzymatic breakdown of the living matter of the tail. The products are then used up as sources of energy.

Q.5. Which pair of limb appear first ?

Ans. The hind limbs appear first.

Q.6. Which environmental factors influence the developmental stages?

Ans. Quality of water in terms of acidity or alkanity and temperature fluctuations.

Investigatory Project 6

Aim : *Study of life history of Mosquito, Fruitfly or Housefly, Cockroach, collection and preservation.*

(a) Requirements for Mosquito (Anopheles or Culex)

Broad mouthed containers and/or any other stagnant water, hand lens, camel hair brush, 70% alcohol, small tubes with corks.

Sources- (1) Collect any pond water, keep it in a dark place, mosquitoes will come and lay eggs.
(2) Flower vases with money plant or other floral display used for decoration purpose often have mosquitoes breeding in the water.

Observations - Larvae are very easy to detect with unaided eyes. As soon as larvae appear, cover containers with net, to prevent the insects from flying away. All stages of life history can be observed with hand lens, picked up by brush and preserved in separate tubes in 70% alcohol. Make a day to day calender and keep scanning the water surface with a hand lens. Note down all changes with refeence to eggs, larvae, pupae and emergence of adults.

Some useful clues are given below :

1. Watch out for eggs, in large clustres (culex) or isolated (Anopheles).
   Egg cluster - of the shape of a raft, each egg of cigar shape as in culex
   or
   Isolated pointed eggs, with air floats as in anopheles. In both cases, hatching takes place within 1-3 days.

2. **Larvae.** Count the number on successive days. Note position of larvae with respect to water surface, i.e. at an angle- in culex or larva is parallel - in anopheles. (To water surface) Respiratory siphon present in culex, absent in anopheles.

   Note changes in size, for larvae undergo moulting, actively swim and feed and increase in size. Larlval period = 3-14 days in culex, and 2-4 weeks in anopheles.

3. Pupal life in both is for about 2-7 days. Pupae are not as active as the Larvae. They are comma shaped, more so in anopheles. Both have respiratory trumpets, broader and shorter in anopheles.

4. **Adults.** Observe resting position. (Reverse of larvae positions) male, female can be distinguished by studying mouth parts under a microscope in fixed forms.

Further investigation can be done with respect to following :

(a) Can change quality of water, with various chemicals of known conc. and observe their influence on life hostory.
(b) Temperature has a very marked influence, on larval and pupal stages. Water temp. can be varied by cooling or warming.
(c) Influence of darkness, and bright light can be seen.

(d) Effect of insecticides, oily substance sprayed on water, can be observed.

(b) Fruitfly (Drosophila)

Requirements-Jam jars, rubber bands, net, hand lens, camel hair brush, sweet over-ripe fruit, preferably bananas, propionic acid.

Sources-Keep some rotten or over-ripe bananas (smashed into a pulp) in an open jam bottle. With a hand lens, watch out for wriggling maggots. Fruitflies lay eggs on these fruits and maggots, appear within 2-3 days. Once you detect the maggots, cover bottle with a piece of cloth, and a rubber band.

Observation-Maintain a day to day calendar, as suggested before and note down appearance of maggots and adults. Eggs are about 0.5 mm. in length, opaque with dorsal side flat and ventral round, can be seen under microscope. Maggots or larvae are visible with the naked eye. Just before pupating, they creep out of the nutrient medium and adhere to the dry glass surface. (Around 120 hrs.).

Young flies soon appear these are slender, light coloured and become stouter and darker while they grow. Thus younger and older generations can be distinguished. Count number of larvae, number of young and old flies.

You can have many more flies, simply by transferring some flies into a fresh, dry bottle. For this, keep the new bottle on top of the old one mouth to mouth and the flies will rise up, quickly place a cover. After transferring, time taken for the next generation can be observed. Provide food in the new bottle.

(**Note-** To prevent fungal growth on food, use propionic acid, 5 cc per 1000 gms. of mashed banana, or a mixture of sodium benzoate and potassium meta bi-sulphite).

Male, female ratios can be observed, as they are easily distinguishable -
(a) Male is smaller in size.
(b) Male has a black tipped posterior end.
(c) Female has a pointed abdominal end with 7 segments in the abdomen, while male has 5 segments.

Other differences are also there, but these can be found out by the student.

On the basis of information given so far, further investigation can be possible with reference to :
(a) Varying conditions of temperature.
(b) Varying conditions of light and darkness.
(c) Varying conditions of food.

E.g., transfer a few flies on to guava, a few on unripe banana, still others on synthetically sweetened food, etc.

Various stages can be preserved in 70% alcohol.

(c) Housefly (Musca domestica)

Requirements- Jam bottles, brush, decaying food matter, small tubes, 70% alcohol.

Source- Keep any decaying matter (cow/horse dung) in a glass trough-keep it in an open place.

(1) Flies will come and lay eggs and you can observe maggots within 2-3 days.

(2) Flies can also be trapped by attracting them towards sweetened food like cotton soaked in milk in a bottle.

Once maggots are seen, cover the bottles as suggested in the previous cases.

Observe larvae. Note down increase in size every 3/4 days. Find out the maximum length reached and other behavioural features of the larvae. Young flies once hatched can be transferred to fresh containers.

(d) Cockroach (Blatta orientalis)

Requiremnts :- A large size empty shoe box, wood shavings, cellophane paper, tongs or long forceps, bread crumbs.

Sources : Kitchen drains, dustbins, store rooms. Cockroaches can be best available at night. Keep some left overs of food, bread crumbs on the kitchen floor near the drain. Use a torch light at the time of collection. With the help of tongs or forceps, first collect 10-12 cockroaches in a jam bottle. Keep the lid shut.

1. Convert the shoe box into suitable place to keep the insects. Pierce some holes all around and on top of the box. Cut out a rectangular piece of cardboard from the lid of the box and fix some cellophane paper on it. Spread some wood shavings , and bread crumbs in the box. Replace the lid.
2. Transfer all the cockroaches into the above box. Use the transparent window to watch and observe their activities, keep the box in a dark , warm place, eg. the kitchen.
3. Once they get used to the box, they will breed if temperature is suitable, ie. in the months of March/April.
4. Observe and make notes on :
   i) Adult features of male and female.
   ii) Cocoons and their features.
   iii) The nymphs and changes that occur in them.

QUESTIONS

Q.1. What is the usefulness of studying development stages of housfly or cockroach ?

Ans. These are common household insects. They are often a nuisance as they contaminate food articles or clothes by acting as vectors. Knowledge of their developmental stages help in controlling them.

Q.2. Are these insects diurnal or nocturnal ?

Ans. Houseflies are found in larger numbers, during the day time, while cockroaches are nocturnal

Q.3. There is a large variety of advertisement regarding baits which are used to kill these insects. What is the basis of this ?

Ans. The 'baits' are actually sugar coated poisonous substances. The insects get drawn towards it, consume the bait and get killed.

Q.4. Why is it so important to reduce both houseflies and cockroaches in the household environment ?

Ans. These insects visit dirty places such as sewage, excreta, sputum, garbage can, as well as our food articles and other household articles. They often transmit germs responsible for diseases such as chslera, gasteroenteritis etc.

Q.5. Cockroaches have wings, but they are not frequently found flying.

Ans. This is because they get their food mostly on the kitchen floors, near the drains or dustbins. They do not need to be air borne as frequently as some others.

Investigatory Project 7

To study development of drug resistance in becteria using antibiotics.

Materials - Antibiotics-Penicillin, Aureomycin, glass ware. The experiment can be perfomed following the technique explained in the core experiment no 14.

You can also purchase sterilized petridishes along with nutrient medium from the chemist shop. Proceed as following steps :

Method-

1. *Preparation of glass ware containing nutrient medium.*

You can transfer nutrient medium in culture tubes, petridishes or conical flask and sterilize in autoclaves / pressure cooker for 30 minutes. Cool to room temperature. Replace sterilized glass ware in a shelf in the laboratory, which has already been rinsed with alcohol to make the environment free from contamination.

2. *Inoculation of bacterial culture.*

You may use bacterial culture from yogurt, or hay grass. The latter can be prepared by keeping few hay straws in a beaker containing distilled water (about 200 ml. of water is enough in a beaker of 500 ml. capacity). Warm up the water slolwly and bring to boil for five minutes. Allow to cool and keep the same for 2 days in a corner of the lab, preferably covered with a filter paper. A white scum willl appear on the surface of the water in the beaker. This is bacterial culture ready for in oculation (known as Bacillus subtilis).

Transfer a loopful of bacterial culture from yogurt or hay culture into culture tube, conical flask or petridish with the help of sterilized inoculation wire.

3. *Preparation of antibiotics solution and transfer into culture medium .*

Once the inoculation of bacterial culture is completed, you can wait for 48 hour; for incubation period. Colonies of bacterial culture will appear on the surface of the nutrient medium. You can treat these colonies with the antibiotics or if you want to hurry your experiment, the treatment with antibiotics can be given on the same day.

Purchase antibiotics from the market in the form of tablets or vials. Dissolve them in distilled water (5 ml.) with the help of inoculation needle, transfer the antibiotics in loopfuls in the culture medium, by touching the medium in culture tube, flask and in case of petridish a loop of 8 can be made by scratching the needle along with antibiotic over the medium.

Leave the whole set up for 48 hours and begin your observations at every 24 hours interval and record. if any marked region of inhibition of culture is developed. The inhibition region varies with the time, concentration and nature of antibiotic used.

Control Experiment - You should set up control experiment without treatment with antibiotics for comparison.

Observations -The development of colonies of bacterial culture is visible in the medium. However, in the experiment set up, a zone of inhibition is seen, indicating the effect of antibiotics, whereas bacterial colonies continue their growth in center of set up.

After having studied for several days, sometimes new colonies emerge around inhibition zone also.

Discussion -Antibiotics have inhibitory effect on the growth of bacterial culture. On this principle several diseases have been controlled. Continuous use of antibotics has not given similar result. This is because the bacterial culture becomes resitant to the antibiotic. Similarly reemergence of the colonies in culture medium after some time explains that the bacterial colonies have developed resistance towards a particular antibiotic in the experiment.

QUESTIONS

Q.1. Why do bacterial culture stop their growth when treated with antibiotics ?

Ans. Antibiotics have property to suppress the growth of mircoorganism.

Q.2. Why an antibiotic cannot suppress the growth of microorganisms after a prolonged use ?

Ans. Because microorganisms become resistant to the effect of antibiotics.

Investigatory Project 8

Aim : *To study the coaguable and non-coaguable proteins present in milk.*

Material - Cow milk, beaker 250 ml., 2 test tubes, funnel, filter paper, renin tablets or small amount of glacial aceticne acid, burr, pipette, Millon's Reagent, nitric acid, powder milk.

Method - Pipette out 20 to 25 ml. of milk in a beaker and allow to boil. When the same is about to boil, mix grounded renin tablet or one teaspoonful of acetic acid. The milk will curdle. Remove the beaker from the fire and allow the solid portion to settle. Filter out the liquid portion (whey) over a funnel. Transfer a small portion of solid material and mix 2 ml. water and few drops of Millons Reagent. Allow to boil and cool. A light yellow colour appears. Mix few drops of conc. nitric acid and look for any colour change. Repeat the same test with whey water (filtrate). You can try the above test with powder milk after dissolving in water.

Observations - Both, the solid and liquid components of milk give yellow precipitation with Millon's Reagent which turns orange when treated with nitric acid.

Results - Positive Protein test conforms its presence in two components of milk.

Discussion -Cow's milk is a rich and complete food. That's why it is recommended as diet for the infants and sick persons. The composition of milk has almost all the required sources of nutrition. The powdered milk is also used as diet for children and adults. A close look of the composition will explain itself-

Composition of Fresh Milk

S.No.	Components	Percentage (%)
1.	Water	87.3
2.	Butter fat	3.8
3.	Casein (coaguable)	2.5
4.	Albumin and globulin (non-coaguable)	0.7
5.	Lactose	5.0
6.	Meneral	0.7

Composition of Powder Milk (Amul)

S.No.	Components	Percentage (%)
1.	Milk fat	26
2.	Milk protein	28
3.	Milk sugar	37
4.	Mineral salts	6
5.	Moisture	3

Powdered mik can be mixed with water in the proportion of 1:41 for the test.

The major portion of protein is casein which is coagulated on heating with renin or acetic acid. When separated by filtration the solidified portion of milk is rich source of casein. In the filtrate portion non-coaguable proteins are collected and they are of soluble form even after treatment with renin. Thus positive protein test is found in two portions. The soluble proteins are albumin and globulin. The milk also contains antibodies for several diseases. These are globulins. Therefore the infants are fed with fresh milk (if mother milk is not in enough quantity). The proportion of coaguable casein protein is much larger then that of non-coaguable proteins like albumins and globulins.

QUESTIONS

Q.1. Name the proteins that are found in milk.

Ans. Casein, albumin and globulin.

Q.2. Generally when milk is boiled, it does not coagulate. But it coagulates when mixed with renin or acetic acid. Why ?

Ans. On mixing with renin (an enzyme) or acetic acid, the pH is lowered and that makes milk protein casein to coagulate.

Q.3. What is the chemical composition of casein ?

Ans. Casein is a protein which has phosphorus attached as the prosthetic group. Hence it is conjugate protein called phosphoprotein.

Q.4. What is coagulation ?

Ans. Proteins solidify as a precipitate or particulate matter from a soluble salt to their insoluble form. This is coagulation and is possible thermally, electrolytically or by bacterial action.

Q.5. What is curd formation due to?

Ans. This is coagulation of the milk protein caesin by the bacterial activity which release acidic substances.

Q.6. Both curd and 'paneer' are coagulated milk proteins. How are they different ?

Ans. Curd has semidigested protein, i.e. the long chain protein polymers are broken down down to short chain structurs. Paneer has all its protein retained as long chain polymers of peptides.

Q.7. When we make curd or paneer from milk, which portions have the soluble (non-coaguable) protein?

Ans. The watery part of curd and the watery part that remains after removing the paneer, contain the soluble proteins.

Investigatory Project 9

Aim : *Study of the efffect of osmotic stress by administration of hyper tonic saline in frog.*

Requirements- Balance, sodium, chloride, measuring cylinders, beakers, injection needle (no. 22-23) syringe of 5 ml., cotton, watch, distilled water, frogs, preferably of approximately same size.

Procedure- Measure the sizes of the frogs. (One person can hold the frog and lay in on the table surface, and the other person can quickly take the measurements. Have some means of lebelling these animals before putting them back to the water through another container with lid. You can use white oil paint; with brush mark them 1, 2, 3, etc. or arrange to keep them in separate cotainers and label the containers. Prepare saline solution of following concentrations -0.6%, 1%, 3%, 5%, 10%, 20% and 30%. Inject (intraperitoneal) different frogs with one of these saline solutions. Can inject two at a time, because if you inject all at once, observation may be difficult. Note time of injection and thereafter, keep a vigilant eye on the behaviour. Observe time taken for death (if death occurs).

Other suggestions - If more animals are available then :

(a) Effect of the same concentrate of saline can be seen in small and big frogs.
(b) Effect of saline can be seen in starved and well-fed frogs.
(c) Same can be seen in frogs kept in lukewarm water and in very cold water.

Various other modifications can be introduced and interesting conclusions drawn on amphibian behaviour towards saline solution in terms of osmotic stress.

QUESTIONS

Q.1. What is the meaning of osmotic stress ?

Ans. All living organisms maintain a certain (optimum) osmotic conditions of their body fluids. This refers to critical levels of salts dissolved in the fluid which is water. Mainteinance of metabolism depend greatly on corret osmotic conditions inside the body. Any disturbance in the salt content or the fluid content beyond the level of tolerance by the organism creates imbalance or disturbance. Such disturbance manifest either in abnormal behaviour or responses. The organism is then said to be in stress.

Q.2. What is isotonic, hypotonic and hypertonic saline ?

Ans. Saline solutions that have solute concentrations same as that of the body fluid (blood) is isotonic. Lower solute concentration in fluid (in comparison to that in the blood) is hypotonic, while a fluid with higher solute concentration in comparison to that in blood is hypertonic.

Q.3. What is the advantage of considering frog for this investigation ?

Ans. Frog lives in fresh water. It is easier to create changes in osmotic conditions of water by simply adding known amount of salts in fixed volumes of water-and determine the exact osmotic condition.

Q.4. Which other animal can you perform this experiment to get identifiable responses ?

Ans. Fishes, earthworms, molluscs.

Q.5. Which internal structures are concerned with osmoregulatory mechanism in all vertebrates ?

Ans. The Nephric tubules in the kidneys.

Investigatory Project 10

Aim : *Locomotion in fish, importance of different fins in balancing and steering the body.*

Requiremnts- Wide mouthed containers or glass troughs, blunt forceps, measuring thread, sharp, small scissors, fish food, hand lens.

Procedure involves having small, easily manageable aquarium fishes and watching them closely, if needed with a hand lens, for details of movements.

Suggestions-(i) First observe normal movements and explain it in terms of as many diagrams as possible. It will be a good idea to measure the fish, and also the fins, with the help of a thread.

(a) Observe the fins during slow movement and while static.
(b) Observe the fins during fast movement.
(c) Observe the fins during turning movement.
(d) Observe the fins during backward and forward movements.
(e) Observe while the fish comes up from the bottom of container to the surface.

(ii) Hold the fish if possible, within the water, with left hand, using a blunt forceps and cut off completely or partially one of the fins. In different fishes different fins can be cut off. Then observe the changes in movement again, thus carefully analyse the exact function of each fin.

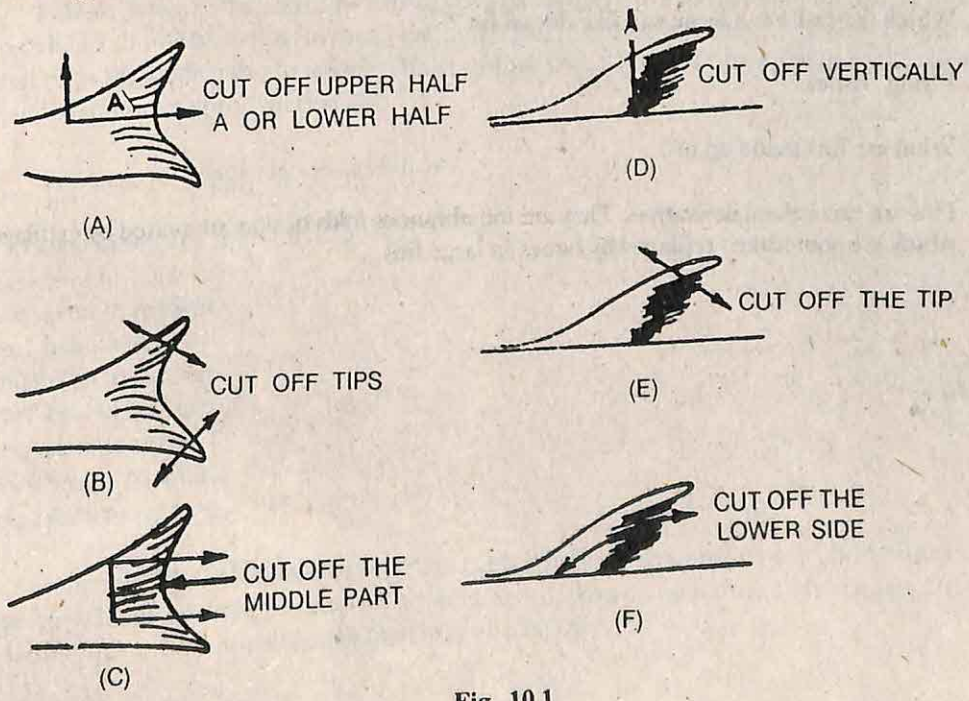


Fig. 10.1.

(iii) With little more patience, you can get further details by giving different kind of cuts to the same fin. For example take tail fin or pectoral fin in different fish and give the cuts at different places.

In this manner, the significance of the shapes and sizes of each fin can be known. Other suggestions:

(a) Reduce or increase depth of water.
(b) Reduce or incrase temp. of water.
(c) Introduce other floating vegetation.
(d) Have more than one type of fish and compare functional details of similar fins.

QUESTIONS

Q.1. Are fins indispensable for swimming in animals ?

Ans. No. because many can swim without fin, and swimming is actually brought about by all the body muscles. Only in some fishes do the fins contribute to swimming.

Q.2. How are fins useful to fishes ?

Ans. Tail fin helps in forward thrust and in changing directions. Dorsal, anal and ventral fins control rolling and yawing. Fishes often roll on their central axis, and yawing is sideways movement. Paired fins such as pectoral and pelvic fins control the pitch, ie. swimming downwards or upwards. They also act as brakes.

Q.3. What happens when fins get injured in the natural course of swimming ?

Ans. Generally injured fins can regenerate.

Q.4. Which fishes have a large sail like dorsal fin ?

Ans. Flying fishes.

Q.5. What are fins made up of ?

Ans. Fins are exoskeletal derivatives. They are membranous folds of skin, supported by cartilagenous rays which are sometimes replaced by bones in large fins.

Investigatory Project 11

Aim : *Effect of alcohol, tea, coffee and nicotine on the heart-beat of frog.*

Heart-beat of an animal remains fixed under optimum conditions, for examples, human heart beats 72 / minute. It is common knowledge that alcohol, tea, coffee and conditions such as stress, excitement affect the rate of heart-beat. Simple investigations to study such effects can be conducted on a frog.

Requirements : Frog, frog ringer solution, alcohol, water extracts of tea, coffee and nicotine.

Procedure : Pith or stun a frog. Pithing may be done by holding the live frog with a duster in the left hand and by piercing a dissecting needle in the frog with your right hand. The piercing should be done in the head region between the eyes. The needle should be pushed backward and forward to make sure that the brain is damaged. Alternatively, stunning can be done by holding a live frog with your right head and lifting the hand of the frog hard against any firm surface like a table.

After the frog has quietened down dissect it open and carefully expose the heart. Remove pericardium and flood it with ringer's solution. Observe the heart-beats for 2 or 3 minutes. Count beats / minute several times and record the average.

Take a piece of dry cotton wad and soak the ringer's solution around the heart. Take another cotton wad soaked in alcohol and squeeze the alcohol gently over the heart, (few drops at a time). Observe change in the heart-beat in the next few minutes. Record the increase or decrease in the heart-beat per minute.

Effects of tea, coffee, nicotine extracts can be observed in a similar manner. But before any of these the frog's heart should be bathed in ringer's solution and the heart-beats allowed to become normal. In case the heart stops responding due to fatigue of the heart muscles, another frog of preferably the same size should be taken for continuing the experiement.

Tabulate your observations as follows :

1. Heart-beat in ringer's solution (control) :
2. Heart-beat in Alcohol ;
   Time taken to recover-
3. Heart-beat in tea :
   Time taken to recover-
4. Heart-beat in coffee:
   Time taken to recover -
5. Heart-beat in Nicotine:
   Time taken to recover-

Other details such as number of drops of each fluid which were just enough to produce a noticeable change in the heart-beat can be counted. You can also make a comparative study in a small frog and a large frog. Different concentration of each fluid may also be tried.

QUESTIONS

Q.1. Why does the heart of the frog keep beating, even after destroying the nervous system ?

Ans. This is because the normal heart-beat is independent of the C.N.S. The heart-beat is myogenic and threfore continues to beat even after the brain is damaged. The myogenic tissue is a specialized part of the heart and it sends out the signals for a rhythmic contraction of the heart. Also, the 0.6% saline keeps the heart tissue living for sometime, even after the animal has been killed.

Q.2. Alcohol, tea, coffee, etc. known to be stimulants. How do they work in a living organism ?

Ans. These chemicals actually stimulate the nervous system. The nerves connected to the heart conduct nerve impulses to the heart muscles and accelerate their working. This results in an increase in the heart-beat for some time. In the above experiment, where the chemicals are directly poured on the heart musceles, they are stimulated to beat faster.

Q.3. What is the important precaution to be considered in this investigation ?

Ans. The heart-beat must be restored to normal before observing the in luence of another chemical.

Q.4. Will you prefer a small frog or a large frog for this experiment ?

Ans. A large frog will be better because it will have a proportionately larger and stronger heart.

Q.5. Why should the chemicals be poured few drops at a time ?

Ans. Too much of a chemical substance poured all at once may be harmful and the heart-beat might stop. By pouring few drop at a time, any change will be easily noticeable.

Investigatory Project 12

Aim : *Effect of fertilizers on seed germination by studying:*

(a) rate of germination,

(b) measuring elongation of hypocotyl and

(c) length of root.

Materials - Chemical fertilizers like the source of NPK - Urea, Ammonium phosphate, Ammonium nitrate, Potassium nitrate. Fairly large size seeds like beans, castor, tamarind, cowpea, petridishes, measuring cylinder, balance and cotton, glass pencil, a thread.

Method - Select healthy seeds of one type at a time. Soak these seeds overnight in water. Prapare different concentration of one chemical fertilizer by dissolving 10 gm. into 100 ml. of water from this stock solutions pour out 10 ml. into 90 ml. water. You will get 1% solution. Pour out 5 ml. of stock solution into 95 ml. water to get 0.5% solution Alternatively, you can weigh different amount of the fertilizer and dissolve in water. It is essential not to use a solution of higher concentration, can you give reason ?

Place a thin lining of cotton in the lower half of the petridish. Sprinkle the solution of different concentration of fertilizer and label the petridish with a glass pencil. Three sets of one concerntration are recommended.

Place 4 seeds of one type into different petridishes containing various concentration of the fertilizers. Keep these petridishes in a dry and dark place (why dark place ?). Take observation every 24 hours till the seeds germinate with radicle emerging out. You can take observation every 12 hours after the emerging of radicle upto the appearance of first leaf. You can record your observation. You have to set one petridish with seeds in water also. Repeat the same experiment with different type of seeds and fertilizer concentration. You will get a good picture of the treatment given to seeds.

Observation - Some of the seeds show good rate of germination in a particular concentration and still some of the seeds to not germinate. The seeds germinate. Yet the size and length of the hypocotyl and root system is not the same in all the concentrations. You can measure the same with a piece of thread. You can record your observations in a table for each seed and concentration of fertilizer. Plot a graph of your results.

Table - Seed Treatment with fertilizer (Urea, Bean)

Concentraton in water	0.5%	1.0%	1.5%	2.0%	3%
Date of germination					
24 hrs. later					
48 hrs. later					
Final elongation of hypocotyl					
Length of root					

Result - The rate of germination of seeds is affected by the treatment of fertilizers. The length of hypocotyl also varies. The root system show profuse growth in fertilizers with different type and concentration.

Discussion -The seed is in the state of dormant embryo. It remains dormant, when favourable conditions are not available. These are suitable temperatre, moisture and air. In some seeds the rate of germinantion of seeds can be accelerated by changing the environment, ie. by mixing, fertilizers, other salts, sugar, light intesity, darkness. All these factors have show some positive and negative effects, on the seed germination. When the fertilizers are mixed in the water of various concentration and types, the seeds of different type shown, different responses. This is an interesting information for the experiment as welll as its application on the greater scale like fields. These minerals have shown their effect on the rate of growth of the plants.

QUESTIONS

Q.1. Why is it recommended to use seeds that germinate by epigeal germination ?

Ans. This is because in this germination the hypocotyl comes above the soil and then it is easy to measure its length.

Q.2. Why is it not recommended to use fertilizers liberally ?

Ans. Excess fertilizers produce toxic effect in the plants.

Q.3. What minerals are supplemented by fertilizers ?

Ans. Nitrogen, potassium, phosphorus and calcium.

Q.4. Why should chemical fertilizers be mixed in the soil ?

Ans. They are injurious to the plant if given directly.

Investigatory Project 13

Experiment - Study of the competition between seeds and crops.

Material - During winter take seeds of pea and gram and during summer take maize and sunflower seeds. Earthenware 6 in. pots, garden soil with enough amount of manure mixed or small flower-bed in a garden.

Method -1. Preparation of flower-bed or pots. Select a small piece of small flower-bed in the school garden exposed to good amount of sunlight, or fill up the mixture of garden soil with manure in pots in multiple of three.

2. Seed sowing - Place about two seeds of each type in the set of pots and six seeds in second set. Or sow the seeds at a distance of 30 cm. in the fower-bed. You should select two patches of flower-bed to one patch. There should be distance of 30 cm. and to second patch of flower-bed these seeds be placed at a distance of 5-8 cm. each.

Water daily and wait till the emergence of radicles and plumules. Once the seedlings are developed, record your obsevations every alternate day by counting number of leaves, length of the flower and any special feature. Label the pots and flower-beds. Record your observation in a table as follows :

Name of the seed	Pot A with 2 seeds of each type	Pot B with 6 seeds of each type
Time taken for the emergence of roots		
How many healthy seedlings after one week		
How much growth after 2 weeks		
How much growth after 4 weeks		
Flowers developed when :		
Total length of the stem		
Total no. of leaves		
Development of fruits		

You can record your observations to cover all the aspect of growing plants.

Observations -The plant need right amount of environmental conditions and spacing for the normal growth. Due to overcrowdings growth of root and shoot is restricted. The healthy plants can survive and weak ones die in the struggle . The similar conditions are also posssible several times in nature . There is struggle for existence due to competition for space, nourishment, light air and several other environmental conditions. That is why the plant do not survive in crowded place except the fittest ones. The nature has arrangements for overcrowding in the plants. You must have noticed that a large number of flowers are borne to a mango tree but the number of fruits developed are few. This is a method to overcome competition within the plants.

The competition is evitable in the gardens also. At seedling stage, several plants grow together. During the course of development the weak ones die. By the time the plant sreach to the stage of flower formation the number is reduced considerably. This is natural competition.

The competition can be intraspecific and interspecific. The first case is common observation. In order to produce healthy plants, it is necessary to shed off unwanted portion like the flowers, leaves fallen off.

In case of interspecific competition, only healthy ones survive in an ecosystem.

In another case environmental competition is also considered. Here, we see in our surroundings that the healthy plants with sufficient abiotic factors are only the ones surviving.

QUESTIONS

Q.1. What is competition ?

Ans. Living organisms share habitats. Their basic needs are similar. For example, plants requrement the same minerals for their growth. It is natural therefore that there is always a competition among plants to obtain the required minerals, from the soil on which they grow. Well developed root systems are better adapted to absorb adequate minerals. There is competition for space, water, sunlight, etc. Plants that grow broad leaves get more sunlight on them.

Q.2. How has competition helped in evolution?

Ans. Organisms with useful adaptations are the "winners" in the competition or in the struggle for existence. These useful variations are perpetuated through reproduction. Thus, these organisms that are fit to survive get automatically selected to survive in nature. The gradual establishment and perfection of the useful characters enable them to be different from the original population, with whom, they no loger interbreed. This is how speciation or evolution of a new species occurs.